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Editorial

Physiologic and Pharmacologic Basis for the Chemotherapy of Psychiatric States

For many years it has been hoped that the central nervous system disturbances considered to be functional in origin might some day be as amenable to medical therapy as are numerous organic disease states. Encouragement has been gained from even such primitive therapeutic procedures as insulin and metrazol® shock, electroconvulsive therapy, inhalation of high concentrations of carbon dioxide and from prefrontal lobotomy. It must be admitted that, even though there has been little scientific basis for their clinical use, dramatic responses to such procedures have been sufficiently frequent to justify continued search for a more physiologic approach to problems of mental disease. The results of observations made in the course of more systematic investigations will be presented to indicate that at least some behavioral phenomena may arise from specific areas of the brain and are subject to modification by surgical procedures or by administration of certain drugs.

Hughling Jackson's concept of encephalic dominance,[1] which postulated that the phylogenetically newer areas of the brain may control and modify the action of more "primitive" brain structures, has perhaps done much to overemphasize the importance of the cortex insofar as control of behavioral and emotional reactions is concerned. Here there must be, he said, a final sensory and motor arrangement that forms the neural substratum of consciousness.

It has been recognized for some time that the diencephalon serves as the source of autonomic nervous system activity, and that visceral expressions of emotion, such as pallor, blushing, increase of blood pressure and pulse rate, are mediated by the hypothalamus. Since many psychiatric syndromes are associated with clinical evidence of autonomic imbalance, as well as with disorders of conceptual formation and attitudes, the existence of neural pathways connecting the cortex and hypothalamus was postulated.[2] It was subsequently observed that sham rage reactions could be induced in cats from which the forebrain had been removed.[3] Since such rage reactions were associated with generalized overactivity of the sympathetic nervous system, it was believed that frontal lobe inhibition of the posterior hypothalamus had been removed.

In a reinvestigation of the experimental production of sham rage, Bard and Mountcastle[4] demonstrated that production of the rage reaction does not depend entirely on the removal of the neocortex. They observed that removal of part of the neocortex may lead to a state of placidity, provided certain portions of the rhinencephalon and at least part of the cingular gyrus were spared. If the amygdaloid complexes and pyriform lobes were removed bilaterally from such a placid animal, a state of ferocity followed. Apparently, inhibitory influences originating in the cingular gyrus in the neocortex and

[1] JACKSON, J. H. Selected Writings of John Hughling Jackson. 2 vols. London, 1932. Edited by Taylor, J.

[2] GRINKER, R. R., INGRAM, W. R. and RANSON, S. W. The hypothalamus—a review. *Psychosom. Med.*, 1: 19–92, 1939.

[3] BARD, P. A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system. *Am. J. Physiol.*, 84: 490–515, 1928.

[4] BARD, P. and MOUNTCASTLE, V. B. Some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behavior. *A. Nerv. & Ment. Dis.*, 27: 362–404, 1947.

in the amygdaloid complex may suppress the mechanisms underlying emotional activity.

Of interest in this connection are the observations of Kluver and Bucy[5] in monkeys in which both temporal lobes were removed and the amygdaloid complexes damaged. These animals, usually quite wild and combative, became tame following this operation. In addition, they exhibited a loss of certain natural fears for objects and reptiles and an increase in sexual activity, usually in the direction of perversion. It seems likely that these behavioral and emotional changes were related to removal of the two hippocampi or of the temporal lobes. Moreover, Kluver and Bucy reported that in a young epileptic patient who had uncontrollable focal psychomotor seizures associated with assaultive-destructive behavior, removal of the suspect temporal lobe was followed by only temporary remission of seizures which recurred with the same uncontrollable behavioral disturbance; but when the remaining temporal lobe was removed, a profound emotional change resulted. The patient became flat in affect, apathetic, showed no interest in anything or any one except his own reflection. He recognized his parents but addressed them as "Mr." and "Mrs." and demonstrated no affection toward them. Sexual perversion and exhibitionism developed. He has remained autistic and is now confined to a mental hospital.

Insofar as the cortex is concerned, the results of studies involving ablation of the prefronto-orbital and cingular cortex[6] suggest that these regions are concerned in the control of emotional activity. Prefrontal activity appears to bestow on the individual the ability to anticipate and plan, and the orbital cortex may function to summon into activity the visceral mechanisms necessary for the performance of the planned somatic motor response. The cingular gyrus, or at least a portion of it, appears to exert some influence on autonomic activity. When it is destroyed experimentally, certain behavioral changes are noted. When it is the site of a disease process, the patient may show disorders of affect. These cortical regions (orbital, cingular and hippocampal, together with the uncus, pyriform area and insula) constitute a large circle of cortex interconnected by fiber systems to a most important brain stem or centrencephalic system which, according to Herrick,[7] includes all the brain except the cerebellum and cerebral cortex and their dependencies. More specifically, from the point of view of functional units, this system is comprised of the thalamus, hypothalamus and reticular formation.

Discovery of the remarkable functional properties of the reticular formation,[8] an area which includes all the gray masses of the tegmentum of the medulla, pons and midbrain which do not belong either to the cranial nerve nuclei, to the relay nuclei of the cerebellar system or to the relay nuclei of the lemniscal systems, has been most important in helping to understand the function of the brain. The physiologic properties of the reticular formation indicate that its activity is related to states of consciousness, sleep or wakefulness.

Penfeld's observations[7] have led him to believe that final functional integration may take place in the centrencephalic system. He points out that such integration cannot take place in the cortex alone since any portion of the cerebral cortex can be removed without producing unconsciousness, and that, when a motor area of the cortex is stimulated, conscious patients are aware that they have not willed the action.

Localized damage to the centrencephalic system may produce loss of consciousness and loss of bodily activity. This may result from a gross lesion, tumor, trauma or inflammation of the third ventricular area or the region slightly posterior to it. A patient with a lesion in this area may lie for weeks in a curious state resembling light sleep and may shift the position of his body as if in sleep. He may occasionally be aroused to speak. He may respond to pain, bright light and noise, and yet he cannot be called "conscious." His sensory motor mechanisms and cortex are intact, yet he shows no signs of reasoning, of awareness, of approval or disapproval, or of volition. This condition may

[5] KLUVER, H. and BUCY, P. Preliminary analysis of functions of the temporal lobes in monkeys. *Arch. Neurol. & Psychiat.*, 42: 979–1000, 1939.

[6] PEELE, T. L. The Neuroanatomical Basis for Clinical Neurology. New York, 1954. McGraw-Hill Book Co., Inc.

[7] PENFIELD, W. Studies of the cerebral cortex of man. In: Brain Mechanisms and Consciousness. A symposium organized by the Council for International Organizations of Medical Sciences. Springfield, Ill., 1954. Charles C Thomas.

[8] MAGOUN, H. W. The ascending reticular system and wakefulness. In: Brain Mechanisms and Consciousness. A symposium organized by the Council for International Organizations of Medical Sciences. Springfield, Ill., 1954. Charles C Thomas.

progress to a state of stupor. The resemblance of such a patient with a high brain stem lesion to one deeply sedated with chlorpromazine and promazine is particularly striking.

These neurosurgical lesions demonstrate that it is possible to produce behavioral and emotional disturbances (rage, placidity or sleep) by interference with specific areas of the brain. There is an increasing body of evidence to indicate that subcortical structures may play a role even more important than that of the cortex in determination of behavioral and emotional characteristics.

Emotional changes can be induced also, of course, by pharmacologic agents. From time immemorial man has been attempting to escape unpleasantness or boredom in his environment by the use of various empirically selected drugs which alter his emotional reactions. The number of such agents is legion; those in common use include alcohol, barbiturates, opiates, coca derivatives and cannabis. These drugs are often unsatisfactory for experimental purposes since, in doses of scientifically interesting magnitude, they affect the sensorium in a typical organic manner producing confusion, impairment of memory, alterations of consciousness and unreliability in reporting subjective experiences. There are, however, a number of drugs which induce in man temporary mental aberrations resembling spontaneous "functional" psychoses. These agents include lysergic acid diethylamide, mescaline and adrenochrome. The mental disturbance induced by these drugs is referred to as a model psychosis because it is accompanied by fear, hostility, feelings of unreality, depersonalization, disturbances of orientation in time and space, and hallucinations. The hallucinatory phenomena presumably differ from those of the genuine psychoses in that they usually occur in the presence of a state of clear insight and with relatively little if any intellectual impairment.

Even before the formal consideration of these ancient and new drugs as "hallucinogens," many investigators were inclined to believe that the functional psychoses were possibly due to an unidentified endogenous intoxicant resulting from an inborn metabolic error.

Fabing[9] has recently reviewed possible metabolic aberrations which might conceivably lead to the endogenous formation of hallucinogenic compounds. One such possible metabolic error concerns the biochemical lesion which may arise from a disturbance in the degradation of epinephrine, a naturally-occurring hormone long known to produce anxiety when administered in minute concentrations. Even on standing, epinephrine may undergo spontaneous oxidation to adrenochrome, an indole-containing compound. It may be of interest to point out that all the known hallucinogens, with the exception of cannabis, contain an indole ring or have the capacity for forming this ring. The injection of 1 to 10 mg. of adrenochrome in volunteer subjects has been reported to produce schizophrenia-like dissociation lasting for as long as four days.[10]

A second and perhaps more interesting possible biochemical aberration is that concerned with the degradation of the amino acid, tryptophan. According to Udenfriend,[11] tryptophan can first be hydroxylated to 5-hydroxytryptophan, which may then be decarboxylated by a specific decarboxylase to form 5-hydroxytryptamine or serotonin. The oxidative deamination of serotonin by monamine oxidase yields 5-hydroxyindoleacetic acid, which is excreted in the urine.

If tryptophan is decarboxylated before its 5-hydroxy derivative is formed, tryptamine results. This compound has been observed to produce catalepsy and negativism in cats,[12] and has also been isolated from the urine of patients suffering from pellagra.

Probably more directly significant than these reactions is the possible role that serotonin may play in abnormal mental processes. It has been known for some time that serotonin is a naturally occurring constituent of nervous tissue, intestine and platelets. It was noted by Woolley and Shaw[13] that most of the hallucinogens were structural analogues of serotonin, and it was hypothesized that their effects might be due to

[9] Fabing, H. D. The abnormal production of indoles as a possible cause of schizophrenia. Read at Midwest Regional Research Conference, A. P. A., Galesburg, Ill., 1955.

[10] Hoffer, A., Osmond, H. and Smythies, J. Schizophrenia, a new approach. II. Results of a year's research. *J. Ment. Sc.*, 100: 29, 1954.

[11] Udenfriend, S. and Titus, E. The 5-Hydroxyindole Pathway of Tryptophan Metabolism. Amino Acid Metabolism, p. 945. Baltimore, 1955. Johns Hopkins Press.

[12] Nieuwenhuysen, F. J. Chronic experimental catatonia produced by intermediate products of metabolism. *Proc. Roy. Acad. Amsterdam*, 39: 1151, 1936.

[13] Woolley, D. W. and Shaw, E. Some neurophysiological aspects of serotonin. *Brit. M. J.*, 2: 122, 1954.

competitive inhibition of serotonin activity. It was shown by Woolley and by Gaddum[14] that these drugs, particularly lysergic acid diethylamide, produce specific inhibition of serotonin action on smooth muscle, functioning as antimetabolites. On the basis of peripheral effects, it was postulated that lysergic acid exerts its central nervous system effects by opposing the action of serotonin. This concept has been reinforced by the observation of Udenfriend[11] that serotonin apparently can be synthesized within the brain, as evidenced by accumulation of the substance following the administration of 5-hydroxytryptophan or of iproniazid, which inhibits the serotonin-destroying enzyme, monamine oxidase.

It may be significant, according to Pletscher et al.,[15] that the serotonin concentration in the brain stem of rabbits is about three times that of the remainder of the brain. These authors' studies on animals suggest that reserpine exerts its effects by modifying serotonin activity. The evidence they present for this concept is as follows: (1) When mice are given serotonin, they become tranquilized, indicating the central site of action of the substance and its ability to penetrate the blood-brain barrier. (2) Serotonin potentiates the effects of hypnotics in a manner similar to that of reserpine. (3) Reserpine administered to dogs induces a marked urinary excretion of 5-hydroxyindoleacetic acid.

They have also pointed out that a short time after administration, reserpine can no longer be found in the brain although serotonin-binding inhibition may persist for as long as forty-eight hours. These observations have led them to postulate that serotonin may function as a neurohumoral synaptic transmitter and may act primarily in the hypothalamic area as a mediator of inhibitory nerve impulses.

A somewhat different concept has been proposed by Woolley[16] who observed that serotonin markedly increases the normal pulsatile activity of rat oligodendroglial cells in culture, with inhibition of this stimulation by antiserotonins. It was suggested that *in vivo* inhibition of serotonin oligodendroglial stimulation may interfere with the hypothetical "stirring" of extracellular fluid and thereby, by implication, may impair transfer of oxygen and substrates to brain cells.

These observations, although extremely stimulating, do not conclusively prove that disturbances of serotonin metabolism are etiologic in the development of naturally occurring functional psychoses. Although it is most probable that lysergic acid and serotonin have antagonistic actions, there is still much disagreement that this is physiologically significant, rather than only incidental to quite different effects of lysergic acid in the central nervous system. Grenell[17] has presented evidence that seems to indicate that lysergic acid acts to inhibit axodendritic synapses in the cortex rather than in the subcortex where serotonin concentration has been shown to be most marked. In fact, it even has been stated[18] that lysergic acid hallucinations may arise in the retina rather than in the brain. There is considerable evidence that lysergic acid does not block all effects of serotonin and that both serotonin and lysergic acid may act to increase synaptic resistance.[19]

Probably the most pertinent objection to the "serotonin hypothesis" is that recently raised by Cerletti and Rothlin.[20] They observed that introduction of a bromine atom into the lysergic acid molecule completely alters its activity in that it loses the property of producing psychic disturbances but retains the strong antiserotonin action on peripheral tissues. The brominated lysergic acid, when administered to man, does not produce signs of mental or psychic disturbances such as are regularly observed after lysergic acid diethylamide, yet effectively antagonizes the bronchoconstrictor, vasoconstrictor and uterine-

[14] GADDUM, J. H. Ciba Foundation Symposium on Hypertension. London, 1953. J. & A. Churchill, Ltd.

[15] PLETSCHER, A., SHORE, P. A. and BRODIE, B. B. Serotonin release as a possible mechanism of reserpine action. *Science*, 122: 374, 1955.

[16] WOOLLEY, D. W. Evidence for the participation of serotonin in mental processes. Session IV, paper 2, Conference on the Pharmacology of Psychotomimetic and Psychotherapeutic Drugs, New York, 1956. Academy of Sciences.

[17] GRENELL, R. G. Considerations on the mechanisms of action of psychotherapeutic and related drugs. Session VI, paper 5, Conference on the Pharmacology of Psychotomimetic and Psychotherapeutic Drugs, New York, 1956. Academy of Sciences.

[18] APTER, J. T. The effects of hallucinogenic drugs on the electroretinogram. Session II, Paper 3, Conference on the Pharmacology of Psychotomimetic and Psychotherapeutic Drugs, New York, 1956. Academy of Sciences.

[19] MARRAZZI, A. S. and HART, E. R. The possible role of inhibition at adrenergic synapses in the mechanism of hallucinogenic and related drug actions. *J. Nerv. & Ment. Dis.*, 122: 453–457, 1955.

[20] CERLETTI, A. and ROTHLIN, E. Role of 5-hydroxytryptamine in mental diseases and its antagonism to lysergic acid derivatives. *Nature*, 176: 785, 1955.

stimulating effects of serotonin. Since brominated lysergic acid does penetrate the blood brain barrier, as indicated by its central sedative effects, it is difficult to conclude that the hallucinogenic effect of lysergic acid results from serotonin inhibition.

Certain psychoses, such as schizophrenia, have been considered by a process of exclusion, to be of functional origin since no anatomic, biochemical or physiologic aberrations could be identified. However, it should be recognized that the amount of energy required for cerebral function is quantitatively so small that measurement by available technics has been impossible or unreliable. Thus it should not be surprising that total cerebral oxygen consumption is reported by Kety[21] to be identical in normal and in schizophrenic patients. Electroencephalographic studies, too, until recently have shown no consistent abnormality in the functional psychoses when the electrical activity of the brain surface alone was explored. However, renewed interest in the activity of the subcortical areas and their role in integration of behavior has led Heath and associates[22] to explore electroencephalographically deeper areas of the brain. Tracings made from septal and hippocampal leads have demonstrated abnormal spikes and slow waves during schizophrenic activity but not during remissions, nor at any time in non-schizophrenic patients. While an approach to localization of the pathophysiology of schizophrenia has begun, the cause of the disorder remains unknown.

It has been widely recognized that larger doses of the classic aliphatic depressants produce a phylogenetically descending depression of the neuroaxis. Hence, to those who placed major emphasis upon failure of cortical integration as the cause of behavioral disturbances, the therapeutic effects of such drugs as the barbiturates have been somewhat less than anticipated. However, if it is considered that behavioral and emotional disturbances may represent lack of synchronization or impaired transmission of centripetal impulses from subcortical centers, then at once a reason for the relative therapeutic failure of aliphatic depressants is suggested, as is also a new therapeutic target.

Recently there has become available a number of drugs which tend to suppress the overt manifestations of psychoses characterized by psychomotor hyperactivity, of anxiety as seen in emotional tension states, addiction withdrawal syndromes, and even artificially-induced hallucinations.

Intensive investigation to determine the mechanisms and sites of action of these agents has yielded much valuable information, some of which has already been discussed. Those drugs which have enjoyed successful clinical application include reserpine, chlorpromazine, promazine and meprobamate. These agents, although causing localized subcortical inhibition, are unique in that even in large therapeutic doses they produce no demonstrable reduction of total cerebral oxygen consumption, in contrast to that seen with large doses of barbiturates. They do, however, with the exception of meprobamate, potentiate the action of relatively small doses of analgesics and aliphatic depressants, such as morphine, meperedine, barbiturates and alcohol. Here, again, there are no detectable changes in total cerebral oxygen consumption.[23]

Investigation of the modes and loci of action of these drugs by electroencephalographic and biochemical procedures has shown that reserpine in therapeutic doses stimulates the alerting mechanism in the reticular formation but apparently blocks transmission of this increased activity, primarily at the level of the posterior hypothalamic nuclei. As previously mentioned, Pletscher and coworkers have shown that in animals the administration of reserpine results in a marked reduction of cerebral serotonin concentration.[15] It has been demonstrated by Marrazzi and Hart[24] that serotonin may inhibit synaptic transmission, and it has been postulated that its depletion by reserpine may overcome this inhibition.

Chlorpromazine and promazine, according to Rinaldi and Himwich,[25] in relatively low concentrations produce inhibition of the alerting mechanism in the reticular formation, the latter

[21] Kety, S. Cerebral circulation and metabolism. In: The Biology of Mental Health and Disease. New York, 1952. Paul B. Hoeber, Inc.

[22] Heath, R. G. Studies in Schizophrenia. Cambridge, 1954. Harvard University Press.

[23] Fazekas, J. F., Albert, S. N. and Alman, R. W. Influence of chlorpromazine and alcohol on cerebral hemodynamics and metabolism. *Am. J. M. Sc.*, 230: 128–132, 1955.

[24] Marrazzi, A. S. and Hart, E. R. Relationship of hallucinogens to adrenergic cerebral neurohumors. *Science*, 121: 365–367, 1955.

[25] Rinaldi, F. and Himwich, H. E. Drugs affecting psychotic behavior and the function of the mesodiencephalic activating system. *Dis. Nerv. System.*, 16: 3–11, 1955.

drug having a more specific depressant effect. On the other hand, electroencephalographic observation by Berger et al.[26] indicates that chlorpromazine and reserpine reduce electrical activity of the thalamus without having a specific effect on such subcortical regions as the hypothalamus and amygdaloid nuclei. These apparently contradictory results may be explained perhaps on the basis of species difference in experimental preparations. Furthermore, it should be mentioned that any correlation between electrophysiologic, biochemical and clinical activity of these drugs is not apparent from current information.

Since the phenothiazine derivatives apparently have no demonstrable effect on serotonin metabolism, such as has been demonstrated for reserpine, another basis for their action must be sought. Grenell et al.[27] have shown that adenosine-triphosphate (ATP) utilization is suppressed by chlorpromazine to the greatest extent in the portion of the brain containing the reticular formation. On the other hand, Brodie[28] has suggested that chlorpromazine may act by inhibiting another neurohumoral transmitter, norepinephrine.

Meprobamate apparently reduces the electrical activity of the thalamus,[29] probably by interfering with thalamocortical and corticothalamic neuronal circuits. The pharmacologic basis for this interference has not yet been elucidated.

These physiologic and pharmacologic observations indicate that the various drugs mentioned act primarily on the centrencephalic system within different subcortical areas, either by modification of neuronal transmission or perhaps by influencing cellular energy production. Clinical observation also suggests that the drugs under discussion act principally on different subcortical areas.[30] The failure of reserpine to antagonize the effects of sympathomimetic substances, such as epinephrine and norepinephrine, as well as its apparently predominant parasympathomimetic activity, indicate that its inhibitory action may be primarily localized to the posterior hypothalamus. Chlorpromazine differs from reserpine in possessing moderate to pronounced adrenolytic and gangliolytic effects, as evidenced by its occasional acute hypotensive action which may be accompanied by marked orthostatic features. The antiemetic effect of chlorpromazine and promazine is further evidence of a somewhat different locus of action from that of reserpine which, in contrast, does not inhibit vomiting induced by copper sulfate or other substances. Meprobamate appears to exert little or no effect on vegetative functions and shows practically no potentiation of central depressants. On the other hand, it causes relaxation of striated muscle and in large doses may abolish some of the deep tendon reflexes.[29]

The neurotoxic effects of the phenothiazine derivatives and active Rauwolfia alkaloids may be considered chiefly as extensions of their pharmacologic activity. This is particularly evident in the appearance of extrapyramidal disturbances when large doses of either reserpine or chlorpromazine are administered over a relatively long period of time.[31] These include motor restlessness (jitters), dystonic muscular activity and pseudoparkinsonism. According to various investigators, tremor may be produced by stimulation of the brain stem reticular formation. Rinaldi and Himwich[25] have shown electroencephalographically that in large doses chlorpromazine produces stimulation of this structure. As previously mentioned, reserpine activates the reticular formation while blocking some of its outflow in the hypothalamus. On the other hand, the antiparkinsonism drugs have been shown by Rinaldi and Himwich[32] to produce a specific depression of the reticular formation. As might be expected, they are antidotal insofar as the management of extrapyramidal manifestations

[26] Berger, F. M., Campbell, G. L., Hendley, C. D., Ludwig, B. J. and Lynes, T. E. Comparative effects of tranquilizing agents on subcortical centers and on serotonin excretion. Session IV, paper 5, Conference on the Pharmacology of Psychotomimetic and Psychotherapeutic Drugs, New York, 1956. Academy of Sciences.

[27] Grenell, R. G., Mendelson, J. and McElroy, W. D. Effects of chlorpromazine on metabolism in the central nervous system. *Arch. Neurol. & Psychiat.*, 73: 347–351, 1955.

[28] Brodie, B. B. Serotonin as a possible neurohumoral agent. Session III, paper 5, Conference on the Pharmacology of Psychotomimetic and Psychotherapeutic Drugs. New York, 1956. Academy of Sciences.

[29] Berger, F. M. The pharmacological properties of 2-methyl-2-n-propyl-1,3-propanediol dicarbamate (Miltown), a new interneuronal blocking agent. *J. Pharmacol. & Exper. Therap.*, 112: 413–423, 1954.

[30] Fazekas, J. F., Shea, J. G. and Sullivan, P. D., Jr. Ataractics in medical practice. *GP.* (In press.)

[31] Ayd, F. J., Jr. Physiologic and neurologic responses to chlorpromazine. Their clinical significance and their management. Read at Midwest Regional Research Conference, A. P. A., Galesburg, Ill., 1955.

[32] Rinaldi, F. and Himwich, H. E. The site of action of antiparkinson drugs. *Confinia Neurol.*, 15: 209–224, 1955.

of both chlorpromazine and reserpine administration is concerned, as well as of some of the other undesirable effects of reserpine. Parenthetically it may be noted that the antiparkinsonism drugs themselves often possess some tranquilizing properties in therapeutic doses.

In view of the locus of action of chlorpromazine and reserpine, evidence of disturbed temperature and appetite regulation accompanying their use should not be unexpected. Most frequently the subject exhibits slight hypothermia; occasionally hyperthermia is encountered. Although increased appetite is most common following the administration of these drugs, anorexia is sometimes observed. It is possible that certain endocrine disturbances, such as lactation and menstrual aberrations, not infrequently accompanying prolonged use of these agents,[31] may be due to interference within the hypothalamic-hypophyseal axis.

The behavioral changes noted following their administration are more closely related to the desirable effects for which chlorpromazine and reserpine are usually administered. Exaggeration of tranquilizing activity may produce drowsiness, emotional depression or withdrawal. The converse may also appear as evidence of the ambivalent action of these drugs; insomnia, anxiety or excitation is occasionally encountered.

These side effects tend to emphasize the existence of a link between involuntary physiologic activity and certain functions of the brain which have been considered to be abstract activities; they also serve to re-emphasize the primary subcortical sites of action of the tranquilizing drugs.

The mechanism of action of the various ataractic drugs is not yet completely understood. They do not cure but they seem to modify the overt manifestations of abnormal mental states, either by interference with neuronal transmission or by depression of cellular energy formation or utilization. The ability of these drugs simultaneously to influence emotional activity and to cause demonstrable physiologic changes within specific areas of the brain may soon help to identify objective lesions in the so-called "functional" psychiatric disturbances.

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Clinical Studies

A Clinical Study of the Effect of Arginine on Blood Ammonia*

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THE association of protein metabolism with hepatic encephalopathies may be traced to the original observations of Hahn[1] in Pavlov's laboratory who described "meat intoxication" in dogs with an Eck fistula. Approximately forty years later Monguio[2] demonstrated that the levels of ammonia were elevated in the blood of a dog with an Eck fistula and meat intoxication, a finding which has more recently been confirmed by Riddell et al.[3] and by DeRiemer et al.[4] By the administration of ammonium salts or of urea, Van Caulaert[5] produced the clinical picture of "hepatic coma" in patients with portal cirrhosis. Shortly thereafter, in the course of an exhaustive study of ammonia metabolism, Kirk[6] demonstrated that the increase in the levels of ammonia in the peripheral blood which followed oral administration of ammonium salts to patients with liver disease was not due to impairment in the formation of urea but to the development of collateral shunts between the portal and systemic veins.

Although a practical method for determining the level of ammonia in the blood was first described by Conway approximately twenty years ago,[7a,b] the importance of disorders of ammonia metabolism in clinical disease has only recently been appreciated. Notably as a result of the increasing use of vascular shunting procedures to ameliorate portal hypertension, extensive literature has appeared on the association of elevated levels of ammonia with hepatic encephalopathies. In addition, the possible toxic effects of ammonia in other diseases are receiving increasing attention. The present concepts of the role of ammonia in clinical syndromes has been summarized in detail in a recent editorial by Bessman.[8]

On the assumption that elevated levels of ammonia that occur in blood in association with certain disease states exert a toxic effect, the therapeutic regimen has included measures designed to reduce the concentration of this metabolite in the blood. Observations in this laboratory on the effect of intravenously administered amino acids on blood ammonia indicated that arginine had a marked capacity to lower experimentally induced elevated levels of blood ammonia.[9] The blood urea nitrogen increased concomitantly with the decrease in blood ammonia, suggesting that the effect of arginine on the blood ammonia was mediated by enhancement of urea production. Because of reports on the use of monosodium glutamate to lower blood ammonia,[10,11] the effect of arginine on experimentally induced ammonia intoxication was compared with that of monosodium glutamate.[12] This study confirmed the superiority of arginine both in protection against ammonia intoxication and in treatment. This property of arginine to decrease significantly blood ammonia levels under experimental conditions suggested a trial of this agent for a similar purpose in clinical disease states associated with elevated blood ammonia levels.

MATERIAL AND METHODS

Fifteen patients† with elevated blood ammonia levels and varying degrees of encephalopathy were studied. The patients could be classified into four major categories with respect to the causes of the increased levels of ammonia in the blood: (1) Exoge-

† Patients were from the University of California Hospital, San Francisco Hospital, Franklin Hospital, Veterans Administration Hospital and the United States Public Health Service Hospital, San Francisco.

* From the Surgical Research Laboratories of the University of California School of Medicine, San Francisco, California. Supported by the Christine Breon Fund for Medical Research.

nous ammonia intoxication from ingestion of ammonium salts or diamox,®[13] or from the intravenous use of ammonium salts to correct disturbances in acid-base balance. (2) Portal cirrhosis in association with an increase in nitrogenous substances within the intestine as a result either of a high protein diet or of gastrointestinal hemorrhage (most commonly due to esophageal varices). (3) Surgical shunting procedures performed for the relief of portal hypertension secondary to intra- or extra- hepatic obstruction. (4) Acute hepatic insufficiency as a result of viral hepatitis.

The solutions of arginine for intravenous administration were prepared by adding 25 gm. of L-arginine hydrochloride* to 400 ml. of pyrogen-free distilled water, followed by autoclaving at 15 pounds pressure for twenty minutes. Immediately before injection, 100 ml. of 50 per cent dextrose were added aseptically, resulting in 500 ml. of a 5 per cent solution of arginine hydrochloride in 10 per cent dextrose, with a pH of 5.9 to 6.1. Each single dose of arginine consisted of an intravenous infusion of 500 ml. (25 gm. of arginine hydrochloride) given over a period of one to two hours.

Samples of blood were drawn prior to the administration of arginine and at intervals thereafter. Heparin was used as an anticoagulant. The concentration of ammonia in the blood was determined by the microdiffusion technic of Conway[14] within five minutes after collection of each sample. The normal levels obtained in this laboratory are 40 to 60 μg. per 100 ml. The urea nitrogen of the blood was determined by the manometric method of Van Slyke and Kugel.[15]

RESULTS

The levels of ammonia and of urea nitrogen in the blood, together with associated pertinent clinical findings prior and subsequent to arginine therapy, are summarized in Table I. In every case a reduction in the level of ammonia in the blood occurred following arginine therapy. The most satisfactory results were obtained in patients with acute ammonia intoxication secondary either to massive hemorrhage or to ingestion of ammonium chloride. In those patients with chronically elevated blood ammonia the response to arginine, although definite, was somewhat more delayed. However, in each case improvement in the mental status of the patient accompanied reduction of the blood ammonia level. In more than half the cases the patients were initially in deep coma and, with one exception, became alert and had a clear sensorium within twenty-four to forty-eight hours following arginine therapy.

Of the three patients with acute yellow atrophy who were given arginine while in deep coma two responded in twenty-four to thirty-six hours with a marked improvement in mental status and eventual recovery. The third patient had malignant disease with metastases to the liver, as well as homologous serum jaundice. She responded only briefly prior to her death.

The clinical and biochemical details of each case in which arginine was used are presented. Two cases that illustrate well the ability of arginine to effect a reduction in the elevated levels of ammonia, as well as the associated clinical response, are summarized graphically in Figures 1 and 2.

CASE REPORTS

CASE I. A. D., a fifty-seven-year old man with known cirrhosis, was treated for ascites and peripheral edema. Ammonium chloride had been used as a diuretic agent for approximately five days. The patient awoke on the fifth morning in a confused state and was admitted to the hospital with a presumptive diagnosis of cerebrovascular accident. The initial blood ammonia level was 167 μg. per 100 ml. An infusion of 25 gm. of L-arginine was given and within ninety minutes the patient's sensorium began to clear and his blood level of ammonia had dropped to 139 μg. per 100 ml. Another 25 gm. of arginine were infused and within ninety minutes the patient was alert and lucid and his blood ammonia had decreased to 76 μg. per 100 ml.

CASE II. J. H., a seventy-one-year old white man with known Laennec's cirrhosis of six years' duration, was being studied in the urologic service because of nephrolithiasis when he had a massive upper gastrointestinal hemorrhage. The patient rapidly became stuporous and confused, and a gross flapping tremor developed. The hemorrhage was controlled by a Sengstaken tube but the patient remained in a semicomatose state. The blood ammonia level twenty-four hours later was 139 μg. per 100 ml. After a two-hour infusion of 50 gm. of arginine the blood ammonia level had dropped to 37 μg. per 100 ml. and the patient's sensorium had cleared remarkably. He was conversing intelligibly and the previously noted tremor had disappeared. No further bleeding occurred with removal of the Sengstaken tube and the patient was prepared for a surgical shunt procedure.

CASE III. C. S., a forty-two-year old white man, was brought to the hospital in deep coma. The patient had had many previous entries because of chronic cirrhosis with recurrent bleeding esophageal varices. Evidence of recent massive upper gastrointestinal hemorrhage was found. A Sengstaken tube was passed, and gastro-

* Obtained from The Nutritional Biochemicals Corporation, Cleveland, Ohio and the California Foundation for Biochemical Research, Los Angeles, Calif.

TABLE I

CLINICAL AND BIOCHEMICAL ASPECTS OF CASES IN WHICH ARGININE THERAPY WAS USED

Case No.	Sex and Age	Diagnosis	Time	Blood		Treatment	Clinical Condition of Patient
				Ammonia Nitrogen (μg. %)	Urea Nitrogen (mg. %)		
I	M, 57	Cirrhosis with ascites and peripheral edema, treated with oral ammonium chloride.	1:30 P.M.	167	19	.....	Confused; flapping tremor.
			1:40–3:10 P.M.	...	..	25 gm. arginine	.....
			3:15 P.M.	139	22	.....	Sensorium clearing; tremor gone.
			3:20–4:40 P.M.	...	..	25 gm. arginine	.....
			4:45 P.M.	76	26	.....	Patient alert; sensorium clear.
II	M, 71	Portal cirrhosis, bleeding esophageal varices.	10:30 A.M.	139	15	.....	Semi-comatose; disoriented.
			10:35–11:20 A.M.	...	..	25 gm. arginine	.....
			11:30 A.M.	54	17	.....	Sensorium clearing.
			11:30 A.M.–12:30 P.M.	...	..	25 gm. arginine	.....
			12:45 P.M.	37	20	.....	Coma lightened perceptibly; patient recognizes family and surroundings.
III	M, 42	Portal cirrhosis, bleeding esophageal varices.	10:00 A.M.	147	15	.....	Patient stuporous.
			10:00–11:20 A.M.	...	..	25 gm. arginine	.....
			11:30 A.M.	116	17	.....	Patient responding to stimuli and clear within 24 hours.
IV	F, 58	Portal cirrhosis, bleeding esophageal varices.	1:00 P.M.	174	12	.....	Patient drowsy, confused, disoriented.
			1:00–2:45 P.M.	...	..	25 gm. arginine	.....
			3:00 P.M.	142	15	.....	Patient alert and oriented; mental status normal by next morning.
V	F, 52	Portal cirrhosis, bleeding esophageal varices.	9:30 A.M.	186	10	.....	Patient confused and drowsy.
			9:35–10:50 A.M.	...	..	25 gm. arginine	.....
			11:00 A.M.	132	13	.....	Mental status improved; however, patient died from massive gastrointestinal hemorrhage.
VI	M, 48	Portal cirrhosis, bleeding esophageal varices.	6/21: 4:00 P.M.	184	27	.....	Patient in deep coma; Cheyne-Stokes respirations.
			4:00–6:00 P.M.	...	..	25 gm. arginine	.....
			6/22: 9:30 A.M.	100	28	.....	Coma lighter; patient's breathing improved.
			9:45–11:30 A.M.	...	..	25 gm. arginine	.....
			2:30 P.M.	62	33	.....	Patient responding to stimuli and continues to improve. Died of exsanguination next morning.
VII	M, 45	Portal cirrhosis, bleeding esophageal varices.	6/14: 3:30 P.M.	80	54	.....	Abnormal mental status; bleeding continues.
			6/15: 8:30 A.M.	193	44	.....	Patient comatose, oliguric.
			9:00–11:00 A.M.	...	..	25 gm. arginine	.....
			12 N	183	46	.....	.....
			1:00–2:30 P.M.	...	..	25 gm. arginine	.....
			4:20 P.M.	152	53	.....	Responding to stimuli with purposeful movements.
			6/16: 10:20 A.M.	128	67	.....	.....
			6/17: 9:05 A.M.	227	88	.....	Bleeding continues; coma deepens; patient oliguric.
			6/18: 10:45 A.M.	258	97	.....	Patient in deep coma; expired from hemorrhage.
			2:20 P.M.	...	..	.....	
XII	M, 35	Homologous serum jaundice, acute yellow atrophy.	5/31: 11:30 A.M.	196	11	.....	Patient in deep coma, with fetor hepaticus, Cheyne-Stokes respirations.
			11:30 A.M.–1:00 P.M.	...	..	25 gm. arginine	.....
			1:30 P.M.	184	13	.....	.....
			1:30–3:10 P.M.	...	..	25 gm. arginine	.....
			3:15 P.M.	176	20	.....	Patient's breathing improved, sensorium began to clear; recognized family next day.
			6/2: 10:00 A.M.	79	13	.....	Alert within 36–48 hours after treatment.

TABLE 1 (*Continued*)

CLINICAL AND BIOCHEMICAL ASPECTS OF CASES IN WHICH ARGININE THERAPY WAS USED

Case No.	Sex and Age	Diagnosis	Time	Blood		Treatment	Clinical Condition of Patient
				Ammonia Nitrogen (μg. %)	Urea Nitrogen (mg. %)		
XIII	M, 24	Homologous serum jaundice.	6/11: 10:45 A.M.	173	9	.....	Patient in deep coma; Cheyne-Stokes respirations.
			11:00 A.M.–1:00 P.M.	...	..	25 gm. arginine	.....
			7:45 P.M.	151	11	.....	Breathing improved.
			6/12: 12:15–2:00 A.M.	...	..	25 gm. arginine	.....
			9:40 A.M.	91	12	.....	Beginning to respond to painful stimuli with purposeful movements.
			10:00 A.M.–12 N	...	..	25 gm. arginine	.....
			2:00 P.M.	53	12	.....	Recognizes name but cannot answer.
			3:10 P.M.	44	12	.....	.....
			4:30 P.M.	53	12	.....	Recognizes parents.
			6/13: 4:00 A.M.	...	..	.....	Conscious; talking in short phrases.
			9:15 A.M.	57	12	.....	Sensorium clearing.
			3:30 P.M.	67	11	.....	.....
			6/14: 10:40 A.M.	30	17	.....	Patient begins to eat; alert; sensorium clearing.
			6/18: 9:00 A.M.	32	15	Protein increased gradually to 100 gm. per day.	No effect from 100 gm. protein diet.
VIII	F, 38	Portal cirrhosis, splenorenal shunt.	1:30 P.M.	161	19	.....	Mild disorientation.
			1:35–3:30 P.M.	...	..	25 gm. arginine	.....
			3:45 P.M.	112	22	.....	Patient alert.
IX	F, 27	Banti's syndrome, splenorenal shunt, episodic stupor.	5/22: 3:00 P.M.	199	9	Unrestricted diet	Essentially alert.
			5/23: 3:00 P.M.	233	9	.....	Confused, lethargic.
			5/24: 7:00–9:00 A.M.	...	..	175 gm. protein diet	.....
			1:30 P.M.	311	8	.....	Inebriated behavior, slurred speech, staggering gait.
			1:30–3:20 P.M.	...	..	25 gm. arginine	.....
			3:30 P.M.	258	11	.....	Vision clearing.
			3:30–4:45 P.M.	...	..	25 gm. arginine	.....
			5:00 P.M.	244	14	.....	Sensorium clearing.
			5/25: 9:30 A.M.	180	11	.....	Patient alert.
			9:30–11:00 A.M.	...	..	25 gm. arginine	.....
			11:30 A.M.	119	12	.....	Patient alert, asymptomatic.
X	M, 39	Portal cirrhosis, portacaval shunt.	9:30 A.M.	174	10	.....	Patient lethargic.
			9:30 A.M.–11:00 A.M.	...	..	25 gm. arginine	.....
			11:15 A.M.	138	14	.....	Patient more alert.
XI	F, 70	Lymphosarcoma with widespread metastases; homologous serum jaundice, acute yellow atrophy (virus A).	2:30 P.M.	157	47	.....	Patient in deep coma.
			2:30–4:15 P.M.	...	..	25 gm. arginine	.....
			4:30 P.M.	129	71	.....	Coma temporarily improved; patient expired next morning in deep coma.
XIV	M, 58	Infectious hepatitis.	6/27: 4:20 P.M.	94	19	.....	Sensorium cloudy, patient disoriented.
			6/28: 10:30 A.M.	123	27	.....	Increasing lethargy.
			11:30 A.M.–1:00 P.M.	...	..	25 gm. arginine	.....
			2:30 P.M.	66	29	.....	Patient's sensorium clearing.
XV	M, 57	Metastatic lymphosarcoma to liver with jaundice and ascites.	6/26: 3:00 P.M	119	..	.....	Patient confused, semicomatose.
			3:15–4:50 P.M.	...	..	25 gm. arginine	.....
			5:15 P.M.	97	..	.....	Some clearing of sensorium.
			6/27: 9:30 A.M.	84	..	.....	Patient alert, conversing with family, sensorium clear.

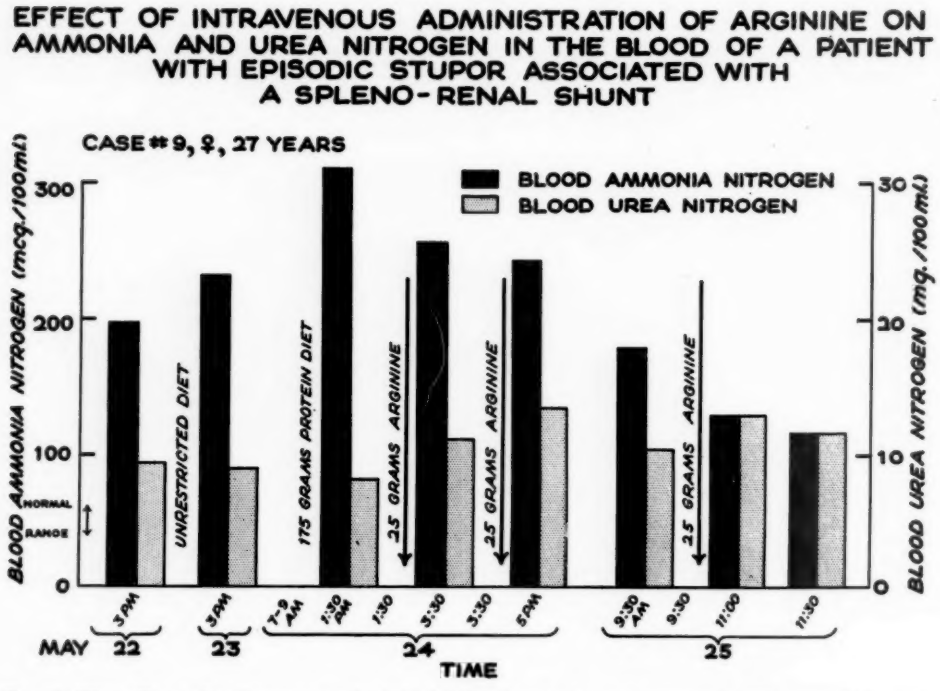

FIG. 1. The effect of intravenous administration of arginine on ammonia and urea nitrogen in the blood of a patient with episodic stupor associated with a splenorenal shunt.

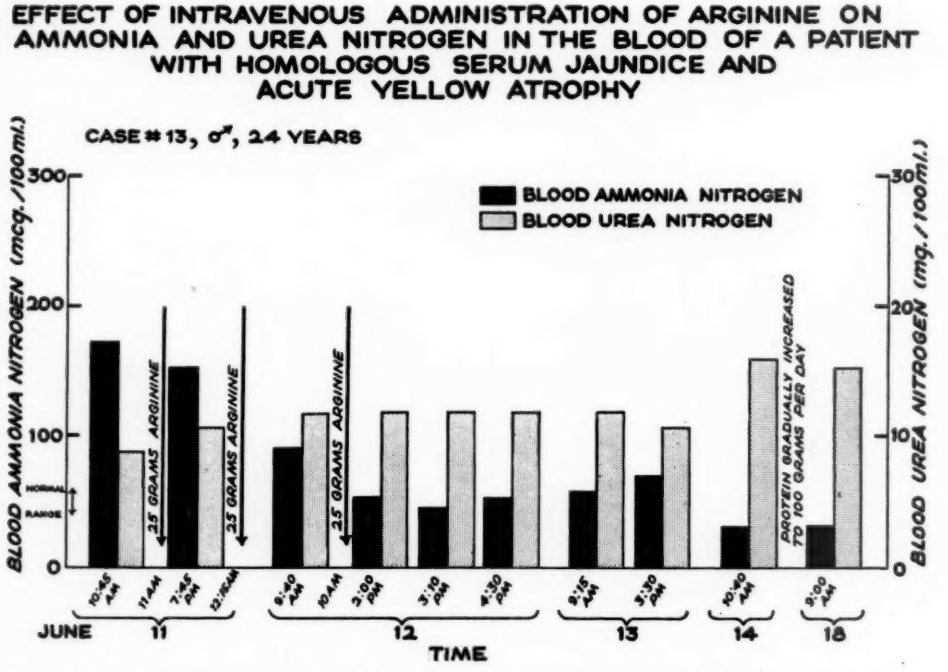

FIG. 2. The effect of intravenous administration of arginine on ammonia and urea nitrogen in the blood of a patient with homologous serum jaundice and acute yellow atrophy.

intestinal lavage was performed. The blood ammonia level obtained on entry to the hospital was 147 μg. per 100 ml. Twenty-five gm. of arginine were administered. Within two hours the blood ammonia was 116 μg. per 100 ml. and the patient began to respond to painful stimuli. Within forty-eight hours the patient's sensorium had cleared and he was prepared for a vascular shunting procedure.

CASE IV. L. D., a fifty-eight-year old white woman with known Laennec's cirrhosis of seven years' duration and ascites of two years' duration, was admitted to the hospital for the first time because of bleeding esophageal varices. The patient had one previous minor episode of hematemesis two months prior to entry which subsided without therapy. A Sengstaken tube was passed to control hemorrhage. The gastrointestinal tract was lavaged and enemas were used to evacuate the blood from within the intestine. However, by the second day the patient became drowsy, confused and disoriented; the blood ammonia at this time was 174 μg. per 100 ml. Twenty-five gm. of arginine were administered and within two hours the patient was alert and oriented as to time and place. The patient continued to improve and no further bleeding occurred. She was prepared for a vascular shunting procedure.

CASE V. M. D., a fifty-two-year old white woman with known cirrhosis of ten years' duration, entered the hospital in preparation for a vascular shunting procedure because of three attacks of upper gastrointestinal hemorrhage within the past year. During hospitalization the patient had a massive hemorrhage from esophageal varices. A Sengstaken tube was passed and the hemorrhage was controlled. However, the patient became confused and lethargic, and a flapping tremor developed. The blood ammonia level was 186 μg. per 100 ml. Twenty-five gm. of arginine were administered intravenously. Within two hours the tremor had disappeared and her mental status was clearing remarkably Four hours later another massive upper gastrointestinal hemorrhage occurred and the patient died of exsanguination.

CASE VI. A. B., a forty-eight-year old man, was brought to the hospital in deep coma with a history of hematemesis that had lasted for three days. A Sengstaken tube was passed and the hemorrhage was controlled. However, as the patient began to respond he became psychotic, pulling out the tube on two occasions while the gastric bag was still inflated. Following this experience bleeding recurred and the patient regressed again into deep coma. The blood ammonia level at this time was 184 μg. per 100 ml. Twenty-five gm. of arginine were administered intravenously, with subsequent decrease in the blood ammonia level when observed the next morning. Another 25 gm. of arginine were given, resulting in further reduction of the blood ammonia level to 62 μg. per 100 ml. and definite signs of clearing as evidenced by improved breathing and response to stimuli. By the next morning the patient was responding with purposeful movements and improved mental status. However, later in the day another acute massive hemorrhage occurred which resulted in the patient's death.

CASE VII. L. R., a forty-five-year old white man, was admitted to the hospital for treatment of upper gastrointestinal bleeding. On admission the patient was lethargic and confused. A diagnosis of bleeding esophageal varices was made. At this time the level of blood ammonia was 80 μg. per 100 ml. A Sengstaken tube was passed but the hemorrhage did not stop and within twelve hours the patient was in coma with a blood ammonia which had increased to 193 μg. per 100 ml. He was also oliguric (blood urea nitrogen 44 mg. per 100 ml.). Following the intravenous infusion of 50 gm. of arginine the blood ammonia level had decreased to 128 μg. per 100 ml., with a concomitant rise in blood urea nitrogen to 67 mg. per 100 ml. The sensorium of the patient had improved perceptibly, with purposeful response to stimuli and improved respirations. However, the patient remained oliguric and continued to bleed in spite of the Sengstaken tube. He died two days later. At the time of death the blood ammonia level had increased to 258 μg. per 100 ml.

CASE VIII. E. B., a thirty-eight-year old white housewife, had had a splenorenal shunt performed for portal hypertension and recurrent bleeding esophageal varices three years prior to the present admission. Since this operation her varices had bled on three occasions. She was admitted to the hospital for treatment of recurrent leg ulcers but because of a mild state of lethargy and disorientation the blood ammonia was determined and found to be 161 μg. per 100 ml. Following a ninety-minute infusion of 25 gm. of arginine her blood ammonia was reduced to 112 μg. per 100 ml.; the patient was subjectively improved and objectively more alert. She has subsequently been placed on a low-protein diet and no further episodes of lethargy and confusion have occurred.

CASE IX. D. Y, a twenty-seven-year old white housewife, was admitted to the hospital with a diagnosis of psychomotor seizure. A splenectomy and a splenorenal shunt procedure had been performed in 1952 for cirrhosis and portal hypertension with congestive splenomegaly. The patient did well until 1954 when she began having episodes of irrational behavior, staggering gait and gross hand tremor, but without loss of consciousness. These attacks would last from one to eight hours after which the patient had no recollection of the event. Because of these attacks she was studied in various clinics and the diagnosis of psychomotor seizures was made. She was then treated with phenobarbital and mebaral® but over a period of one year there was no decrease in the frequency or in-

tensity of the attacks. On admission to the hospital her blood ammonia was found to be 199 μg. per 100 ml. A tentative diagnosis of episodic ammonia intoxication resulting from an unrestricted protein diet was made. During the initial period of hospitalization, without any dietary restriction, the blood ammonia rose to 233 μg. per 100 ml. and the patient began to have symptoms such as slurred speech, staggering gait and blurred vision. At this time an electroencephalogram was obtained which revealed changes compatible with those that are said to occur when blood ammonia levels are elevated. The following morning 175 gm. of protein were given to the patient in order to precipitate an attack. Approximately two hours after the ingestion of this high protein meal the patient had pronounced slurring of speech and she no longer could read average newsprint. Her behavior was described as that of one in a state of inebriation. Mental confusion continued and by 1:30 P.M. of the same day her blood ammonia had risen to 311 μg. per 100 ml. Therapy was then instituted, using 50 gm. of intravenously administered arginine. By the following morning the blood ammonia was reduced to 180 μg. per 100 ml. Her mental status had cleared completely; she was able to walk without staggering and could read the newspaper with ease. Another 25 gm. of arginine were administered intravenously and her blood ammonia level was further reduced to 119 μg. per 100 ml. within two hours.

Phenobarbital and mebaral therapy was discontinued and a low protein diet with orally administered antibiotics was prescribed at the time of discharge from the hospital. She has since been examined in the neurology clinic on two occasions with no evidence of further attacks.

CASE X. J. A. is a thirty-nine-year old white man who had had a portacaval anastomosis performed four years before for recurrent bleeding esophageal varices secondary to portal hypertension. The patient was being studied in the hospital for an unrelated condition and blood ammonia levels were determined to assess the effect of an unrestricted diet. The blood ammonia level was found to be 174 μg. per 100 ml. The patient was then given 25 gm. of arginine after which the blood ammonia was reduced to 138 μg. per 100 ml. He was then given a low-protein diet and has since followed an uneventful course. The moderate blurring of vision that had been present prior to the reduction of protein intake has disappeared completely.

CASE XI. F. B. is a seventy-year old white woman who had had a thoracic laminectomy performed because of the presence of lymphoma of the thoracic spinal cord. The operative findings revealed metastatic lymphosarcoma with apparently widespread disease. She received two units of blood during the procedure. Three weeks after the operation anorexia, nausea and vomiting developed and the patient was observed to be icteric. Within two days the icterus was marked and her liver, which had been tender and palpable three to four fingerbreadths below the right costal margin, could no longer be felt. A diagnosis of homologous serum jaundice with acute hepatic necrosis was made. By the following day the patient was in coma and her liver could now be percussed only two fingerbreadths above the right costal margin. The patient was oliguric and the blood ammonia level was 157 μg. per 100 ml. with a blood urea nitrogen of 47 mg. per 100 ml. Twenty-five gm. of arginine were administered intravenously. The blood ammonia was reduced to 129 μg. per 100 ml. and there was a concomitant rise in the blood urea nitrogen to 71 mg. per 100 ml. The level of coma remained unchanged and the patient died the following day, at which time the blood ammonia had increased to 193 μg. per 100 ml. At postmortem examination the liver weighed only 600 gm. and a large metastatic lesion filled approximately one-half of the remaining atrophic liver.

CASE XII. A. M., a thirty-five-year old white male electrician, sustained an electric flash burn to the face and in the course of treatment received one unit of plasma. Approximately eighty days later the patient began having "flu-like" symptoms and soon observed dark urine and clay-colored stools. Five days later he was admitted to the hospital. At this time the serum bilirubin was 10 mg. per 100 ml., prothrombin time was less than 10 per cent, and liver function was grossly reduced. The edge of the liver was just palpable below the right costal margin. Within twenty-four hours the patient's neurologic state had deteriorated to deep coma, with classic fetor hepaticus and progressive decrease in the size of the liver. A diagnosis of acute hepatic necrosis secondary to homologous serum hepatitis was made and the patient was treated daily with oxygen therapy, vitamin K (10 mg. intramuscularly) and 20 units of ACTH in 10 per cent dextrose administered intravenously. In addition, 2 gm. of neomycin® were given each day by tube. The coma persisted and the blood ammonia level was found to be 196 μg. per 100 ml. At this time 50 gm. of arginine were administered. Four hours later the blood ammonia had decreased to 176 μg. per 100 ml. and coma was perceptibly less. By the following morning the patient was responding to audible and tactile stimuli and by noon he recognized his family and was conversing in short phrases. Thirty-six hours after arginine therapy the blood ammonia level was 79 μg. per 100 ml. and the patient was alert and taking nourishment voluntarily. The edge of the liver was now down to the costal margin. Within a week the dietary protein intake was gradually increased to 100 gm. per day and convalescence proceeded uneventfully.

CASE XIII. G. E., a twenty-four-year old white man, entered the hospital with a diagnosis of homologous serum hepatitis and a history of one week of anorexia, nausea, vomiting and jaundice. On the morning after

entry the patient became confused, disoriented, manic and hallucinatory. The patient was treated daily with oxygen therapy, vitamin K (10 mg. intramuscularly) and 20 units of ACTH in 10 per cent dextrose administered intravenously. In addition, 2 gm. of neomycin were given each day by tube. His mental status worsened and at 3:00 P.M. the next day he became comatose and Cheyne-Stokes respirations developed. The blood ammonia was 173 μg. per 100 ml. Twenty-five grams of arginine were administered and the blood ammonia level was reduced to 151 μg. per 100 ml. Breathing improved so that it was no longer Cheyne-Stokes in character, and coma lessened somewhat. Another 25 gm. of arginine were infused. By the next morning the blood ammonia level had dropped to 91 μg. per 100 ml. and the patient was responding to painful stimuli with purposeful movements. A third dose of arginine was administered and by that afternoon the blood ammonia levels were between 44 and 53 μg. per 100 ml. The patient was now able to recognize his name but unable to answer. By 4:00 A.M. the next morning his sensorium had cleared remarkably and he was able to speak in short phrases. From this point on the patient's sensorium continued to clear. The blood ammonia level dropped to 30 μg. per 100 ml. and the patient was started on a low-protein diet. The diet was gradually increased to 100 gm. of protein within a five-day period and blood ammonia level remained at 32 μg. per 100 ml., indicating major improvement of hepatic function which was substantiated by the results of tests of liver function. Further progress was uneventful and the patient was discharged from the hospital.

CASE XIV. P. T., a fifty-eight-year old Filipino mess man with no history of having received blood or plasma or of having been inoculated in the last six months, entered the hospital deeply jaundiced (serum bilirubin, 21 mg. per 100 ml.). A diagnosis of infectious hepatitis was established and after routine supportive therapy of one week's duration the patient began showing signs of confusion, disorientation and lethargy. The blood ammonia level was 94 μg. per 100 ml. By the next morning lethargy and confusion had increased and the blood ammonia level had risen to 123 μg. per 100 ml. In order to prevent what was interpreted as impending coma, 25 gm. of arginine were infused. The blood ammonia level was reduced to 66 μg. per 100 ml. within three hours and the patient's sensorium cleared.

CASE XV. H. K., a fifty-seven-year old white man with a diagnosis of widespread lymphosarcoma, was being studied at the hospital for possible palliative therapy. The patient had a diffusely enlarged, nodular liver, jaundice and ascites. He was becoming more confused during hospitalization and subsequently lapsed into coma. His blood ammonia level was 119 μg. per 100 ml. Twenty-five grams of arginine were administered; the blood ammonia was reduced to 97 μg. per 100 ml. and some clearing of his sensorium occurred. By the following morning the patient was alert, conversing with his physician and family, and the blood ammonia had dropped to 84 μg. per 100 ml. Because of widespread disease no further arginine therapy was given and within twenty-four hours the patient lapsed into coma again. Three days later he died of generalized metastases of lymphosarcoma.

COMMENTS

The property of the amino acid arginine, to effect a reduction in the ammonia of the blood may be attributed to its role as a precursor of ornithine in the Krebs-Henseleit urea cycle.[16] The reactions involved in this cycle, as pictured schematically in Fig. 3, include additions that have been made since the original work of Krebs and Henseleit. Carbon dioxide and ammonia in the presence of adenosine triphosphate (ATP) react to form carbamyl phosphate.[17] Through a process of "transcarbamylation" the carbamyl group is transferred to aspartic acid to form carbamyl aspartic acid,[18] which in turn donates the carbamyl group to ornithine, forming citrulline and regenerating aspartic acid. (Fig. 3, reactions 1, 2 and 3.) Next, arginosuccinic acid, a compound resulting from the condensation of citrulline and aspartic acid (reaction 4)[19–21] is split into arginine and fumaric acid (reaction 5). Finally, arginine is converted in the liver under the influence of arginase to ornithine and urea (reaction 6). Fumaric acid ultimately forms oxaloacetic acid which by transamination regenerates aspartic acid for use in the formation of arginosuccinic acid (reactions 7, 8 and 9). Four amino acids are therefore directly concerned with the cycle by which urea is formed: aspartic acid, arginine, ornithine and citrulline. Aspartic acid may be formed readily by transamination of oxaloacetic acid, a component of the citric acid cycle, and ornithine and citrulline arise from arginine. This last amino acid is thus an essential and possibly a rate-limiting precursor of the other components of the urea cycle.

The clinical use of ornithine or citrulline to reduce blood ammonia has not been reported. However, Greenstein[22] has reported that neither citrulline nor ornithine was as effective as arginine in protecting against experimentally induced ammonia intoxication in rats.

Formation of urea is normally the major route for removal of ammonia. This reaction should, therefore, be the most efficient to reduce elevated levels of ammonia in the blood. The fact

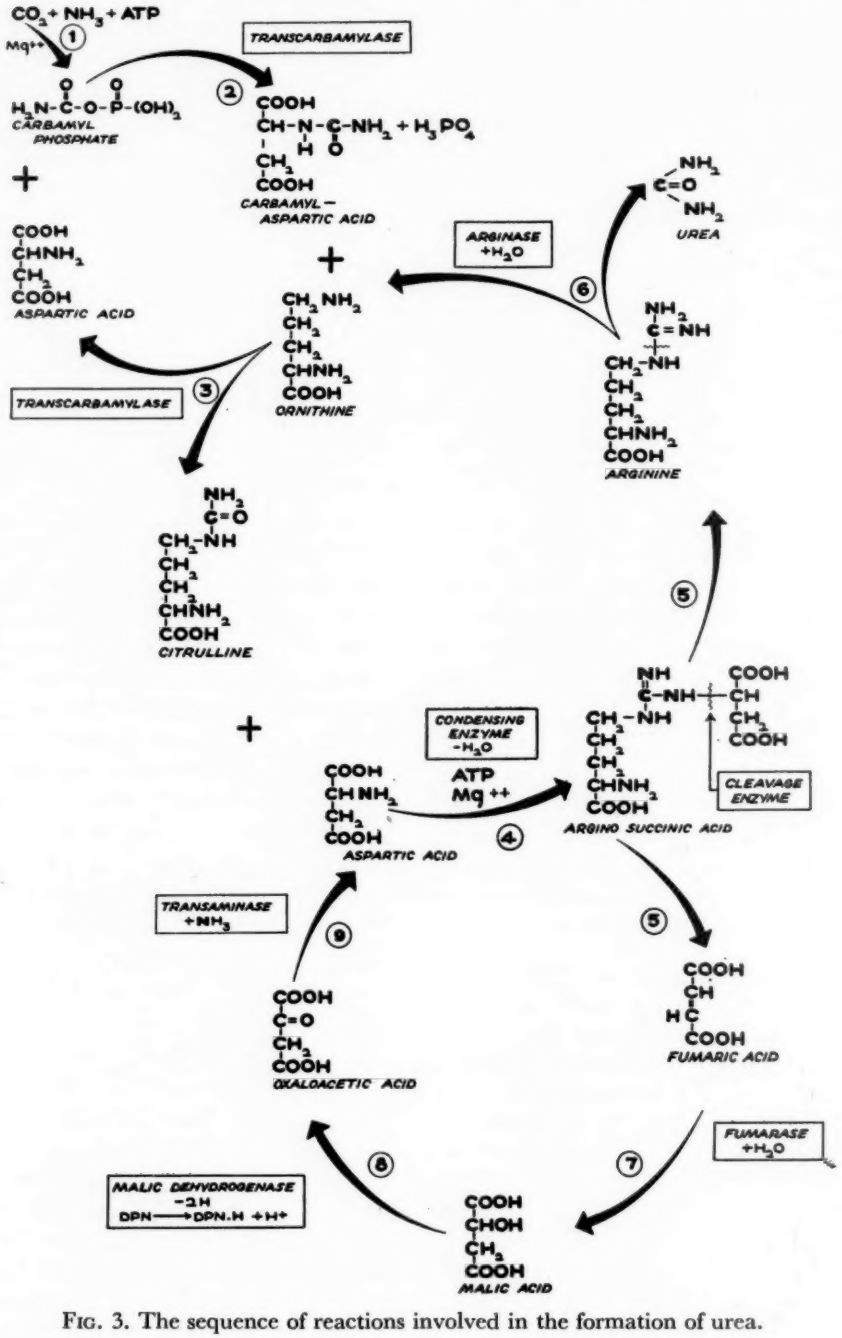

FIG. 3. The sequence of reactions involved in the formation of urea.

that formation of urea is an exclusive function of the liver poses the question of its effectiveness in the presence of marked disease of the liver. In reply it may be pointed out that experimental removal of 80 to 90 per cent of the liver was not found to produce any significant effect on urea synthesis,[23,24] and that in perfusion experiments using livers from dogs poisoned by the use of toxic agents it was demonstrated that damage to the liver must be extreme before production of urea fails.[25] These experimental findings are confirmed by the clinical experience reported here in which reduction of blood ammonia and concomitant increase in blood urea nitrogen could be demonstrated even in the presence of severely damaged liver parenchyma.

The use of arginine or any other substance to lower the blood ammonia can be considered a means of control of only the immediate problem of ammonia intoxication. Unless the underlying

disease process that originally contributed to the production of the toxic state can be relieved the improvement can only be temporary. For this reason treatment of ammonia intoxication in a potentially reversible disease should be the most satisfactory application of this therapy. Such a situation obtains in patients with normal liver function who have been given toxic doses of ammonium salts, and some patients with acute viral hepatitis also may be in a similar category. From the results in two such cases it appears that satisfactory control of ammonia intoxication is an important element in the treatment of the acute phase of the disease. The situation may be analogous to control of potassium intoxication in the treatment of acute renal insufficiency. The two patients with fulminating viral hepatitis had lapsed into deep coma with pronounced fetor hepaticus, Cheyne-Stokes respirations and markedly altered hepatic function associated with liver atrophy; within twenty-four hours after the blood ammonia levels had been lowered by the use of arginine, respirations improved and subsequent clearing of the sensorium occurred.

It is of interest and perhaps of prognostic significance that in both cases the blood urea nitrogen was abnormally low during the acute phase of the disease and that a rise to normal levels accompanied the fall in blood ammonia and the clinical improvement. Within two weeks both patients were taking a normal diet, jaundice was decreasing and liver function was returning to normal. Gradual increases in protein intake up to 100 gm. per day were permitted as soon as the patients were able to take nourishment. Tolerance to the ingested protein was tested by occasional measurement of the blood ammonia, which remained normal throughout the period of convalescence.

Increased amounts of ammonia are introduced into the portal circulation when ammonium salts or a high-protein diet are ingested. Extensive hemorrhage into the gastrointestinal tract may produce a similar result. In the presence of impaired hepatic function and/or collateral communications between the portal and systemic veins so common in cirrhosis, the high concentrations of ammonia present in the portal blood bypass the hepatic barrier and the ammonia content of the peripheral blood may then increase to toxic levels. Surgically produced shunting procedures also foster this source of ammonia intoxication. While efforts to reduce the blood ammonia are an important aspect of the immediate phase of the treatment of ammonia intoxication, measures must also be taken to decrease or eliminate the sources of ammonia within the intestine. These include dietary restriction of protein; discontinuance of administration of ammonium chloride, of resins containing ammonia, or of diamox; and efforts to control gastrointestinal bleeding by the use of the Sengstaken tube. After control of hemorrhage has been achieved the blood within the gastrointestinal tract must be removed with the aid of gastric lavage, enemas, and milk of magnesia introduced via the tube periodically throughout the day. Neomycin (2 gm. per day) by tube may also be used to reduce the bacterial flora within the intestine. If all of these measures are successful the blood ammonia will remain within a safe range after it has been decreased by the intravenous administration of arginine. Otherwise it will tend to increase again, with recurrence of toxic signs.

Further study is necessary for adequate assessment of the importance of elevated blood ammonia levels in various disease states. It can be anticipated that, as further experience is obtained with an agent such as arginine, which can effectively lower the blood ammonia level, a definite appraisal of the pathologic importance of this metabolite can be made.

SUMMARY

1. The ability of intravenously administered L-arginine hydrochloride to decrease the blood ammonia was investigated in fifteen patients with elevation in blood ammonia and varying degrees of encephalopathy associated with several disease entities.

2. In every case there was a reduction in the ammonia levels of the blood following arginine therapy. Improvement in the mental status of the patient accompanied the fall in blood ammonia. Two of three patients with severe viral hepatitis who were given arginine while in deep coma responded in twenty-four to thirty-six hours with marked clinical and biochemical improvement and eventual recovery.

3. The decrease in the level of ammonia in the blood that occurred after administration of arginine was always accompanied by a significant rise in the blood urea nitrogen, suggesting that the effect of arginine on the blood ammonia may be mediated through its role in the urea cycle.

4. Further clinical experience with substances such as arginine which can effectively lower the blood ammonia should serve to delineate more clearly the pathologic importance of ammonia intoxication in clinical disease.

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Cerebral Metabolism in Hepatic Insufficiency*

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STUDIES dealing with neurologic disturbances associated with hepatic insufficiency have been confined chiefly to attempts at identifying the neurotoxic substance or substances responsible for the condition. For the most part, attention has been directed to the concentration of ammonia and other nitrogenous or sulfur-containing compounds in the arterial[1–3] and cerebral venous blood.[4] It is believed that these substances may alter the activity of certain enzyme complexes concerned with intermediate cerebral metabolism.[4] In the present study, cerebral hemodynamics and oxygen utilization of patients with varying degrees of neurologic dysfunction associated with hepatic insufficiency were investigated to determine whether or not changes in the functions mentioned could be correlated with alterations in blood ammonia and pyruvate levels or with electroencephalographic activity.

METHOD

In all patients there was clinical and laboratory evidence of hepatic insufficiency, the result of advanced cirrhosis; seven of the patients had Eck fistulas. The subjects were divided into three groups according to the presence and severity of neurologic manifestations. Group I was composed of patients with advanced hepatic insufficiency but without obvious clinical evidence of cerebral dysfunction. Group II comprised patients demonstrating lethargy, somnolence and/or confusion as cerebral manifestations of hepatic insufficiency; and Group III consisted of patients in hepatic coma.

Scheinberg and Stead's modification[5] of Kety and Schmidt's procedure[6] for the determination of cerebral blood flow was applied in this study. The gas mixtures utilized were those described by Kety and Schmidt.[6] The oxygen content of the blood was determined by the manometric technic of Van Slyke and Neill.[7] Mean arterial pressure (MAP) was obtained directly from the femoral artery by means of a damped aneroid manometer.[8] Blood ammonia levels were determined by a microdiffusion-nesslerization method modified from Seligson[9] on freshly drawn blood from the femoral artery and the jugular bulb. Blood was drawn without stasis into a previously calibrated 2 cc. syringe, and 1 cc. specimens were ejected into each of two Seligson microdiffusion vessels within fifteen to forty-five seconds after withdrawal. In all cases a water blank was run with each pair of arterial and venous samples.

A standard curve plotting the concentration of ammonia nitrogen against optical density was prepared using a Coleman Universal spectrophotometer. The maximum range of spectrophotometric readings for the standard samples with the blank set at 0 was 0.038 to 0.044 for a 1 μg. and 0.073 to 0.077 for a 2 μg. sample. The maximum difference between any pair of values for the blood samples reported was less than 10 per cent of the mean of the pair.

Pyruvic acid was determined by the method of Lu.[10] Blood was drawn from the femoral artery and jugular vein without stasis and precipitated as rapidly as possible.

Electroencephalograms were recorded on a six-channel Grass electroencephalograph in the conventional manner.

RESULTS

Table I presents the findings in the subjects of group I. When compared to values previously observed in normal subjects of approximately the same age range,[11] the data disclose no significant change in cerebral vascular resistance but a significant reduction ($p < .05$) in cerebral blood flow, cerebral oxygen utilization and arteriovenous oxygen difference. The average value for mean arterial pressure was within normal limits. In seven of twelve cases in which arterial blood ammonia levels were determined, the values were significantly elevated above normal. In all patients the concentration of ammonia was the same or only slightly lower in

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TABLE I

CEREBRAL METABOLISM OF ALERT PATIENTS WITH HEPATIC INSUFFICIENCY

Subject	Age	CBF	$CMRO_2$	CVR	MAP	AVO_2	Art. NH_3	Ven. NH_3	Art. Pyruvic	Ven. Pyruvic
1	38	53.5	2.6	1.5	80	4.94	2.1	2.0	...	...
2	42	34.9	2.6	2.4	82	7.56	1.8	1.5	1.8	1.6
3*	65	42.6	2.1	2.2	93	5.00	0.4	0.4	...	...
4*	65	44.3	1.9	2.1	95	4.19	...	...	...	...
5	40	44.8	2.0	1.8	82	4.46	...	...	...	...
6	44	36.1	2.6	3.5	125	7.26	...	...	...	...
7	53	49.1	2.9	2.3	115	5.97	...	...	...	...
8	55	52.7	2.0	1.8	95	3.87	1.2	1.1	1.6	1.5
9	58	38.6	2.5	2.3	87	6.56	2.5	2.3	1.8	1.6
10	59	55.5	2.4	1.3	74	4.37	...	...	...	...
11	62	54.9	1.6	2.1	115	2.98	...	...	...	...
12	51	32.1	2.1	2.3	74	6.45	...	...	...	...
13	65	43.3	2.0	1.8	77	4.66	3.9	3.7	1.4	1.3
14	46	55.8	1.8	1.1	64	3.26	1.8	1.6	2.5	2.4
15	70	45.9	3.8	2.5	117	8.19	...	...	...	...
16	60	44.1	2.1	1.5	67	4.87	1.7	1.4	2.3	2.1
17	34	52.0	2.8	1.8	92	5.39	0.6	0.5	2.1	1.8
18*	43	54.8	2.1	1.6	87	3.80	0.8	0.7	1.4	1.2
19*	43	62.8	2.0	1.5	91	3.26	1.1	0.8	...	...
20	42	44.8	2.5	2.0	88	5.66	2.2	2.0	1.7	1.5
Mean Values										
	52	47.1†	2.3†	2.0	90	5.14†	1.7	1.5	1.8	1.7

Note: In this and the following tables, CBF signifies cerebral blood flow in cc./100 gm. of brain/min.; $CMRO_2$, cerebral oxygen consumption in cc./100 gm. of brain/min.; CVR, cerebral vascular resistance in mm. of Hg/cc. blood/100 gm. of brain/min.; MAP, mean arterial blood pressure in mm. of Hg; AVO_2, cerebral arteriovenous oxygen difference in vol. %; Art. NH_3, arterial ammonia level in μg./ml.; Ven. NH_3, venous ammonia level in μg./ml.; Art. Pyruvic, arterial pyruvate level in mg. %; and Ven. Pyruvic, venous pyruvate level in mg. %.

* Patients with Eck fistulas.

† Significant, $p < .05$, when compared to values obtained for normal subjects; i.e., CBF, 54; $CMRO_2$, 3.3; AVO_2, 6.

the cerebral venous blood than in arterial blood. In nine subjects in whom blood pyruvate levels were determined the mean values were higher than normal. The cerebral arteriovenous pyruvate difference was of the same magnitude and direction as that noted in normal subjects.

Table II presents the findings in fifteen subjects (group II) manifesting mild to moderately severe central nervous system disturbances associated with hepatic insufficiency. The findings for cerebral blood flow, vascular resistance and mean arterial pressure were not significantly different from those in subjects in group I; the mean value for cerebral oxygen utilization was, however, significantly reduced ($p < .05$). When blood ammonia and pyruvate levels were determined, the results were essentially the same as noted in subjects in group I.

The data for sixteen patients in hepatic coma are presented in Table III. When compared with the results in patients in group II, the data disclose no significant change in arterial blood ammonia levels, rate of cerebral blood flow, cerebral vascular resistance, cerebral arteriovenous oxygen difference or cerebral oxygen utilization. Except for the significantly elevated blood ammonia levels ($p < .02$), significantly reduced cerebral oxygen utilization ($p < .01$) and mean arterial pressure ($p < .01$), the values are essentially the same as noted in patients in group I.

Electroencephalograms were recorded for four patients in the mentally-alert group. Two records were normal, one showed borderline fast and slow low voltage activity, and one (Fig. 1) showed medium voltage slow activity (basi-

TABLE II

CEREBRAL METABOLISM WITH MODERATE CEREBRAL DYSFUNCTION AND HEPATIC INSUFFICIENCY

Subject	Age	CBF	$CMRO_2$	CVR	MAP	AVO_2	Art. NH_3	Ven. NH_3	Art. Pyruvic	Ven. Pyruvic
1	41	48.8	2.1	1.2	59	4.32	2.3	2.2	1.5	1.4
2	59	41.5	1.8	1.7	72	4.43	...	...	...	...
3	40	55.2	2.6	1.0	57	4.63	1.0	0.7	...	...
4	40	39.1	1.3	2.7	105	3.23	...	...	...	...
5	63	28.3	1.1	3.0	86	3.79	...	...	...	...
6	54	24.2	1.3	3.7	90	5.44	...	...	...	...
7	63	23.2	1.8	2.2	50	7.91	...	...	...	...
8*	51	54.2	2.2	2.2	120	4.01	1.5	1.4	...	...
9	54	72.8	1.7	0.8	58	2.34	2.2	2.0	...	...
10	55	37.3	1.8	1.9	69	4.76	2.0	1.8	2.0	1.8
11	62	59.8	1.6	2.1	125	2.70	...	...	...	...
12	40	37.6	2.2	2.1	79	5.88	...	...	...	...
13	76	40.8	1.8	1.5	61	4.35	2.4	2.2	...	...
14	60	27.0	1.0	1.8	49	3.87	2.6	2.2	4.2	4.0
15	42	38.8	1.8	1.8	70	4.51	1.3	1.0	2.1	1.9
Mean Values										
	53	41.9	1.7†	2.0	77	4.41	1.9	1.7	2.4	2.3

* Patients with Eck fistulas.

† Significant, $p < .05$, when compared to values obtained for alert patients with hepatic insufficiency (Table 1).

TABLE III

CEREBRAL METABOLISM DURING HEPATIC COMA

Subject	Age	CBF	$CMRO_2$	CVR	MAP	AVO_2	Art. NH_3	Ven. NH_3	Art. Pyruvic	Ven. Pyruvic
1	42	51.7	2.0	1.5	80	3.97	2.5	2.2	...	...
2	52	58.9	1.5	0.8	46	2.60	3.7	3.6	...	...
3	47	36.4	1.9	1.8	64	5.19	....	2.2	...	...
4	59	40.4	1.8	2.0	82	4.52	....	...	...	...
5	27	28.7	1.7	3.2	92	6.04	....	...	...	...
6	35	41.6	1.6	1.3	54	3.77	4.3	4.0	3.2	3.1
7	47	37.8	1.6	1.4	54	4.36	2.4	2.3	2.5	2.4
8	76	35.5	2.0	1.6	56	5.68	1.4	1.2	2.1	1.9
9	76	29.4	1.7	1.6	46	5.79	1.9	1.7	1.0	0.9
10	55	37.9	0.9	1.5	55	2.31	2.6	2.4	...	...
11*	51	45.3	1.9	2.5	120	4.11	3.9	3.6	...	...
12*	42	41.9	1.2	1.1	47	2.96	....	...	...	...
13	59	43.7	0.9	1.9	84	2.12	....	...	...	...
14	65	41.2	1.8	1.6	66	4.32	....	...	...	...
15	65	31.4	1.2	1.8	56	3.68	1.3	1.1	3.2	...
16	60	32.0	1.2	1.4	45	3.66	2.3	2.1	3.0	2.7
Mean Values										
	54	39.6	1.6†	1.7	65†	4.07	2.6‡	2.4	2.5	2.2

* Patients with Eck fistulas.

† Significant, $p < .01$, when compared to values obtained for alert patients with hepatic insufficiency (Table 1).

‡ Significant, $p < .02$, when compared to values obtained for alert patients with hepatic insufficiency (Table 1).

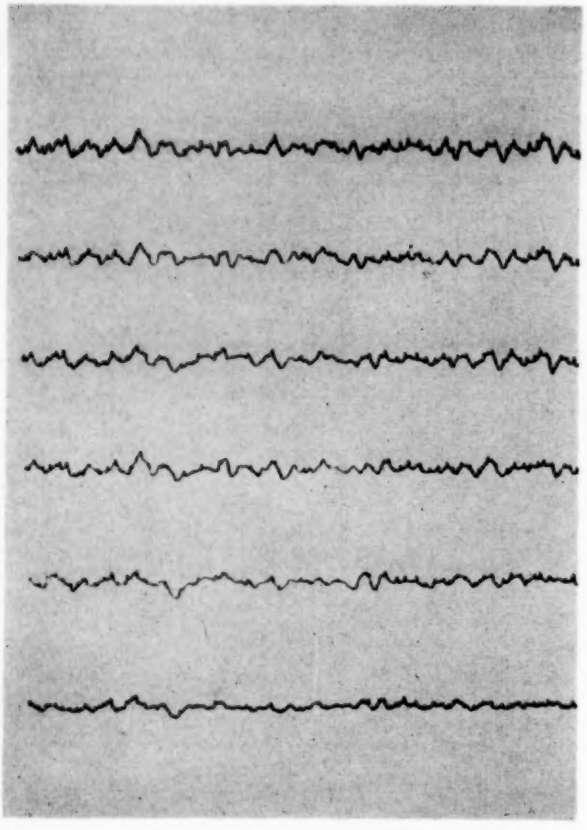

FIG. 1. Electroencephalogram of alert patients with hepatic insufficiency.

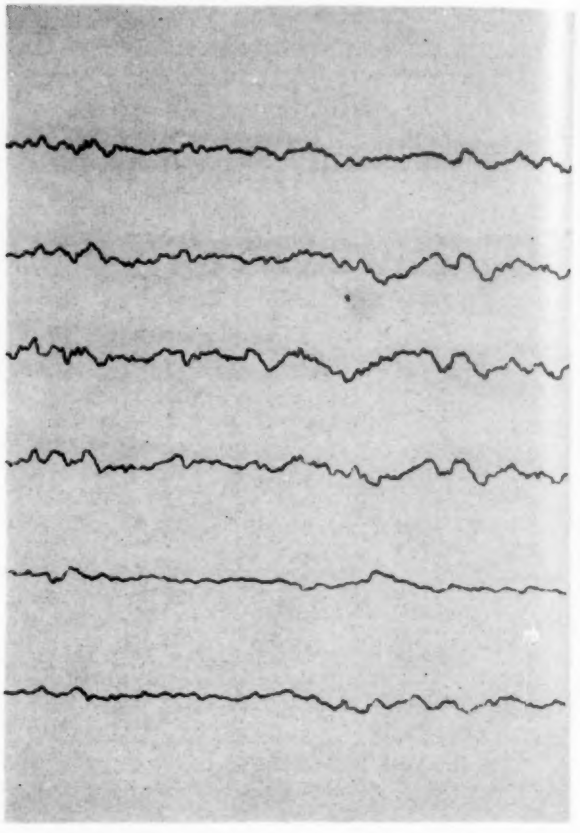

FIG. 2. Electroencephalogram of patients with moderate cerebral dysfunction and hepatic insufficiency.

cally 6–7/second, but with some 1½–3/second activity).

Records were made in twelve patients in group II with impending hepatic coma. One record showed diffuse low voltage fast activity; four records showed borderline slow and fast (mixed) activity and three records showed abnormal slow activity (1½–7/second). Two records (Fig. 2) showed abnormal slow waves with superimposed fast activity. Of the twelve records in this group, seven contained 4–7/second (theta) waves.

Electroencephalographic studies were made in twenty-five patients in deep hepatic coma (some of whom are not otherwise represented in the present study). All the records were abnormal and all showed some degree of slowing. Nine records demonstrated ½–7/second activity, nine showed ½–4/second waves, and seven showed 5–6/second waves. Ten records disclosed high-voltage, irregular, slow, disorganized rhythm (Fig. 3) and four of these also showed high-voltage, fast, spiking bursts. Sixteen records showed voltages higher than normal, and there was definite suppression of normal components in eight of these records.

COMMENTS

The finding of a significant reduction from the normal values for cerebral oxygen consumption and cerebral arteriovenous oxygen difference in subjects with advanced hepatic insufficiency but without obvious clinical evidence of neurologic disturbances (group I) was not anticipated. It is, of course, possible that careful psychologic evaluation of these patients might have disclosed evidence of cerebral dysfunction. Since practically all patients in the present study were chronic alcoholics, the reduced cerebral oxygen utilization might be attributed to cerebral deterioration from prolonged ethanol consumption. It should also be recognized that although the value of 3.5 cc. of oxygen/100 gm. of brain/min. represents the average rate of cerebral oxygen utilization in normal adult subjects, the minimum oxygen requirements of the brain for satisfactory function have not been established. It has been previously demonstrated that there

may be a significant reduction in the rate of cerebral oxygen utilization, as occurs with age, without evident cerebral dysfunction.[11] It is therefore possible that a gradual impairment of cerebral oxidative reactions resulting from hepatic insufficiency may reduce cerebral oxygen consumption to relatively low levels before impressive clinical abnormalities appear. The average critical rate for cerebral oxygen utilization, the rate at which evidence of cerebral dysfunction becomes manifest, has not been determined as yet; it would appear from the present study that this rate is somewhat less than 2.2 cc. of oxygen/100 mg. of brain/min. in patients with hepatic insufficiency.

The significant difference in the reduction of oxygen utilization in subjects in group II and III from subjects in group I indicates clearly a further depression of cerebral enzymatic activity associated with the development of neuropsychologic manifestations of hepatic insufficiency. The lack of a significant difference of cerebral oxygen utilization between subjects of group II and group III suggests that impairment of oxidative reactions may reduce the rate of total cerebral oxygen utilization to relatively low levels before appreciable functional abnormalities appear and that further increments of reduction, supposing a critically low level of metabolism had been reached, may be too minute to be measurable by available technics and yet produce profound deterioration of function. It is further possible that discrepancies between levels of function and total cerebral oxygen consumption may result from regional metabolic impairment. For example, interference with metabolism of large but functionally relatively unimportant areas of the brain might produce a rather large decrease in total cerebral oxygen consumption with little clinical evidence of neurologic change. Localization of the inhibiting factor to small but functionally important structures of the brain might produce profound cerebral dysfunction with negligible further decrease in total cerebral oxygen utilization.

The depression of cerebral oxygen utilization associated with hepatic insufficiency cannot be entirely attributed to an elevated ammonia concentration or its increased uptake by the brain since the changes were not significantly different between subjects with and without central manifestations of hepatic insufficiency (group I and group II). The significantly elevated ammonia level in the comatose group cannot be taken as conclusive evidence that it is responsible for the comatose state, as will be indicated later.

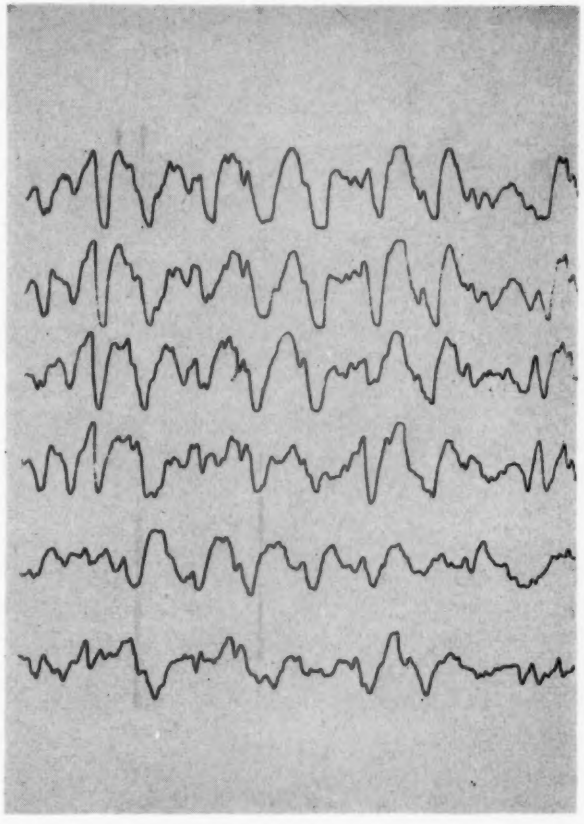

FIG. 3. Electroencephalogram of patients in hepatic coma.

On examination of the cerebral uptake of ammonia by correlating cerebral arteriovenous differences with rate of cerebral blood flow, we have been unable to confirm the previously reported high arteriovenous ammonia difference or an elevated rate of cerebral uptake in patients in hepatic coma.[4] In ten normal subjects, we noted that the ammonia arteriovenous differences and rate of uptake by the brain were of the same order as those noted for the subjects in the present study. In any event, arteriovenous differences of any substance cannot definitely be assumed to indicate alterations in metabolism unless blood flow determinations are simultaneously estimated.

It is also apparent from our data that there is no good correlation between the blood ammonia level and the neurologic manifestations of hepatic insufficiency or the cerebral oxygen utilization since the values for subjects in groups

I and II were not significantly different, and in some instances the arterial blood ammonia values for patients in group III were lower than those observed in group I. It has been postulated that blood ammonia levels may not reflect cerebral intracellular concentrations because of the activity of a system which rapidly binds ammonia by formation of glutamine from glutamic and ketoglutaric acids.[12] However, in the presence of high blood ammonia levels the activity of this or any other ammonia-binding mechanism would have to be accelerated to prevent high intracellular ammonia concentrations (unless there was a marked decrease in cell-membrane permeability for ammonia); the normal rate of cerebral ammonia uptake by our subjects militates against such acceleration, indicating that the variable blood ammonia levels reflect similarly variable cerebral intracellular ammonia concentrations. The present studies do not rule out ammonia as a possible etiologic agent in the development of hepatic coma, but certainly do not indict this substance as the sole responsible factor.

The variation in the arterial blood ammonia level may to some extent depend upon the rate of hepatic blood flow as well as the metabolic efficiency of the liver. It has been demonstrated that in shock without hepatic disease the blood ammonia may be elevated.[13] and it is recognized that in patients with hepatic insufficiency coma may often follow rupture of esophageal varices.[14] In our subjects in coma the significant reduction of mean arterial pressure may well have aggravated liver dysfunction by causing a further reduction in hepatic circulation and impairment of mechanisms for the removal of ammonia.

The significance of the elevation of blood pyruvate frequently noted in patients with hepatic insufficiency and in coma is difficult to evaluate. The metabolic abnormality is not likely to be due to a lack of thiamin or to failure of its phosphorylation. All the subjects in the present series received large amounts of vitamin B_1 prior to the observations. Moreover, the symptom complex in no way resembles clinical neurologic syndromes (such as Wernicke's or Korsakoff's) recognized as resulting from thiamin insufficiency. The diminished rate of cerebral oxygen utilization in hepatic coma itself indirectly indicates a disturbance in carbohydrate metabolism. Since blood glucose levels were within normal limits, the reduction in cerebral oxidation must be ascribed to failure in carbohydrate utilization. The accumulation of pyruvate suggests a disturbance of the pyruvic oxidase system which is concerned with the oxidative decarboxylation of keto acids (such as pyruvic and ketoglutaric acids). Among other effects, specific energy derived from such oxidative reactions is required in the reductive amination of glutamic acid (in part produced by conjugation of ammonia with ketoglutaric acid) to form glutamine. Therefore, impairment of keto acid oxidation may be partly responsible for the elevated blood ammonia levels seen in hepatic insufficiency as well as for accumulation of blood pyruvate. It must be kept in mind that in spite of the large oxygen requirement of the brain, implying large participation in the total carbohydrate oxidation of the body, the increase in blood pyruvate and ammonia seen in hepatic coma may well be due entirely to impairment in metabolism of many other tissues. This is especially probable in view of continued cerebral ammonia and pyruvate uptake during hepatic coma. However, the unique obligatory dependence of the brain upon carbohydrate oxidation as its important source of energy would make it almost the first structure to demonstrate by evident dysfunction any impairment in intermediate carbohydrate metabolism.

The electroencephalographic changes in general paralleled the changes in cerebral oxygen utilization in the various groups and perhaps more closely reflected the extent of the clinical disturbance than did the cerebral oxygen utilization.

SUMMARY AND CONCLUSIONS

Cerebral hemodynamic studies, blood ammonia and pyruvate determinations and electroencephalograms were made in patients with hepatic insufficiency resulting from advanced hepatic cirrhosis and with varying degrees of cerebral dysfunction. Cerebral oxygen utilization was depressed even in those subjects with no clinically evident neurologic disturbances but was further reduced in the presence of coma. It appears probable that the reduction in cerebral oxygen consumption in hepatic insufficiency occurs gradually, beginning some time before neurologic deficits become evident.

Blood ammonia and pyruvate levels were usually elevated but the rate of their cerebral uptake was within normal limits. In this study there was no satisfactory correlation between

arterial blood ammonia levels and cerebral oxygen utilization or the clinical neurologic state.

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The Serum Alkaline Phosphatase in Chronic Infiltrative Disease of the Liver*

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THE finding of an elevated value for serum alkaline phosphatase activity in the presence of a normal or nearly normal serum bilirubin concentration can be of importance in the differential diagnosis of disease of the liver. The rise in serum alkaline phosphatase activity in certain diseases of bone and of the biliary tract is well recognized and the majority of adults with a very high serum alkaline phosphatase activity are found to have Paget's disease, metastatic carcinoma or biliary obstruction.[1–3]

The purpose of this article is to call attention to a smaller group of diseases including tuberculosis, sarcoidosis, systemic lupus, Hodgkin's disease and amyloidosis in which the serum alkaline phosphatase activity may be elevated in the presence of a normal or slightly elevated bilirubin. This disproportion between the serum alkaline phosphatase activity and bilirubin concentration is not usually seen in cases of extrahepatic biliary obstruction. Similar pathologic changes are found in all these diseases of diverse etiology, suggesting that the chemical pattern described is related to an infiltrative granulomatous process common to the entire group. These patients in whom intrahepatic biliary obstruction probably exists will be contrasted with those in whom the obstruction is extrahepatic.

We have included only those patients in whom the majority of the bilirubin determinations were below 2 mg. per cent and the serum alkaline phosphatase activity was greater than 15 Bodansky units. Patients with transient elevations of serum alkaline phosphatase activity seen during the recovery phase of infectious hepatitis and infectious mononucleosis have been excluded. In the latter diseases, especially in the young, the serum alkaline phosphatase activity often remains elevated after the bilirubin has returned to normal. Insofar as possible, patients with disease of the bone have been excluded on the basis of roentgenographic or postmortem information. Eleven of our patients with chronic elevation of serum alkaline phosphatase activity and six similar cases from the literature are presented in Table 1. Data from the eleven patients studied by us are plotted in Figure 1 along with comparable data on fifty-four patients with disease of the common bile duct reported in the literature.[3]

TABLE 1

Case No.* and Patient	Diagnosis	Serum: Alkaline Phosphatase (Bodansky units) Minimum	Serum: Alkaline Phosphatase (Bodansky units) Maximum	Serum: Maximum Total Bilirubin (mg. per cent)
Cases in this Series				
I, B. C.	Sarcoid, tuberculosis	15	46	0.8
II, J. G.	Sarcoid, tuberculosis	15	47	1.8
III, D. S.	Sarcoid	20	50	1.1
IV. P. M.	Systemic lupus, tuberculosis	20	81	0.8–5.0†
V, F. G.	Hodgkin's disease	21	98	0.8–7.4‡
VI, O. C.	Systemic lupus	6	41	0.8
VII, S. G.	? Systemic lupus	23	37	1.1
VIII, R. R.	Tuberculosis, sarcoid	12	68	4.2§
IX, S. S.	Amyloid disease	93	96	1.6
X, I. N.	Sarcoid	7	31	0.8
XI, J. A.	Hodgkin's disease	4	30	0.8
Cases from the Literature				
Spain[7]	Amyloid disease	21, 23		No icterus
Riley[8]	Sarcoid	17		No icterus
Ross[9]	Sarcoid	42		1.8
Shay[10]	Sarcoid	86		1.5
	Sarcoid	22		0.6
	Sarcoid	66		0.7
Klatskin[11]	Sarcoid	57		4.0
Sharnoff[12]	Amyloid disease	30.7		No icterus

* Cases I, IX and X have been previously reported.[4–6]
† Three of twelve determinations exceeded 2.0 mg. per cent.
‡ Single terminal determination.
§ Single initial determination.

* From The Department of Medicine, The Johns Hopkins University and Hospital.

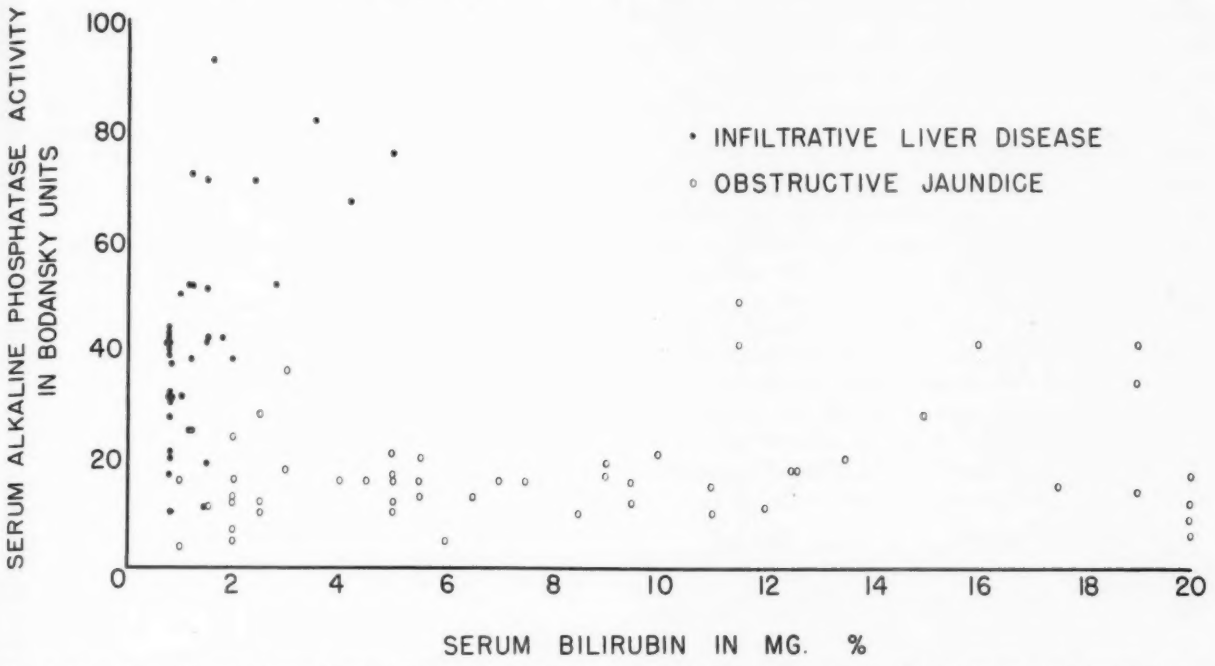

FIG. 1. Data from Cases I through XI in Table I (solid dots) are plotted along with comparable data on fifty-four patients with disease of the common bile duct reported by Gutman, et al.[3] (open circles). The disproportion between phosphatase and bilirubin characteristic of the group with infiltrative disease of the liver is clearly demonstrated.

Seven cases representative of the disease entities responsible for this syndrome have been selected for presentation. Two of the cases not abstracted have been presented elsewhere.[5,6] In addition to these several patients with infiltrative disease of the liver, an unusual case (W. T.) of isolated biliary obstruction has been reported for purposes of comparison. It will be noted that these patients often presented difficult diagnostic problems.

CASE I. B. C. (J. H. H. No. 587942), an eighteen year old Negro woman, was first seen because of hepatosplenomegaly and lymphadenopathy. The lesions of sarcoid were found in a lymph node and in a punch biopsy specimen of liver. Acid-fast organisms which killed a guinea pig were isolated from one of several gastric washings. There was no other evidence of active tuberculosis. The patient was treated with ACTH and has had a remission.

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1951				
Oct. 19	46.5	0.8	3+	10.5
Oct. 26	45.4	...	...	....
Nov. 12	33.0	0.8	2+	11.3
Nov. 19	23.2	...	2+	4.8
Nov. 27	16.4	...	1+	6.0
Dec. 4	33.9	...	0	5.5
Dec. 7	36.6	...	0	6.0
Dec. 18	15.7	...	3+	14.0
1952				
Jan. 8	33.2	...	3+	13.0
Aug. 22	43.2	0.8	3+	18.5
Sept. 9	38.8	...	2+	16.5
Oct. 6	38.9	...	...	....
Nov. 11	41.5	0.8	2+	15.5
1953				
May 14	37.5	0.8	...	....

CASE II. J. G. (J. H. H. No. 587207), a sixty-four year old Negro man, was first seen in 1951 complaining of dyspnea of one year's duration and pruritus of seven months' duration. The liver was percussed a full handbreadth below the costal margin. Small papules were seen on the skin and one large cervical node was found and a biopsy specimen taken. Histologically the lymph node was typical of sarcoid but the skin lesion

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1951					
Oct. 12	42.8	1.6	1.0	3+	3.4
Nov. 8	47.3	...	...	...	...
1952					
Jan. 15	42.7	1.8	1.2	3+	3.2
Jan. 16	43.8	...	...	...	...
Jan. 31	37.9	1.2	0.8	3+	2.3
Feb. 21	27.0	0.8	...	...	...
June 16	15.0	0.8	...	0	1.2
Oct. 28	24.2	...	...	...	...
Apr. 10	22.0	...	...	2+	2.6
1953					
Oct. 23	17.4	0.8	...	2+	4.4

was consistent with sarcoid, tuberculosis or other granuloma. Enlarged hilar nodes were noted on x-ray of the chest. The serum alkaline phosphatase was elevated. The tuberculin skin test was positive in dilution of 1:100. A biopsy specimen was taken of a second lymph node; tubercles with caseation were noted and acid-fast organisms were stained. A punch biopsy specimen of the liver revealed miliary tubercles without caseation or acid-fast organisms. It was concluded that the patient had both sarcoid and tuberculosis. He was treated with streptomycin and para-aminosalicylic acid for eight months, with a 20 pound weight gain and a marked decrease in liver size. The patient has been observed for three years and is now essentially asymptomatic. He weighs 50 pounds more than on admission to the hospital, his liver has receded to the costal margin and the serum alkaline phosphatase is approaching normal levels.

CASE III. D. S. (J. H. H. No. 457501), a forty year old Negro woman, died in 1955, seven years after she was first seen with cervical adenitis. In 1948 a biopsy specimen of a cervical node was taken and the histological diagnosis of tuberculosis was made. Films of the chest showed mediastinal widening and hilar adenopathy on the left. The patient was hospitalized in a tuberculosis sanatorium but after three months all cultures of sputum and lymph node remained negative and she was discharged.

In October, 1952, the patient was admitted with clubbed fingers, edema and dyspnea. The liver was enlarged and scleral icterus was noted. Cultural studies for tuberculosis were all negative; there was no reaction to 1:100 old tuberculin, but second strength P.P.D. gave a positive reaction. A second cervical node was examined and showed caseous necrosis. Culture of the node was again sterile.

During the next two years severe pulmonary insufficiency developed with cor pulmonale. At the time of her final admission in October, 1954, the patient was described as a chronically ill, dyspneic and orthopneic woman. The heart was enlarged to the anterior axillary line, the pulmonary second sound was accentuated and a gallop rhythm was heard. The liver was three fingerbreadths below the costal margin. The spleen was not felt. X-ray of the chest showed bilateral apical fibrosis compatible with tuberculosis or sarcoid. During the subsequent three months the course was one of increasing pulmonary insufficiency and cardiac failure terminating in death.

At autopsy the liver weighed 1,150 gm.; the hepatic, cystic and common bile ducts were patent. The gallbladder was normal. There was early central scarring of the type seen in cardiac cirrhosis but the liver was otherwise normal on microscopic examination.

CASE IV. P. M. (J. H. H. No. 569753), a forty year old white woman, was first seen in 1951 for treatment of systemic lupus erythematosus. During eight years prior to admission the patient had a recurrent facial eruption and systemic symptoms had appeared during the last six months. There was evidence of involvement of the pleura, pericardium, synovia and kidneys; retinal cytoid bodies were described. L.E. cells were present. The chief problem was that of renal failure of the nephrotic type with massive edema, hypoproteinemia and uremia. Because of the renal disease steroids were withheld during the first two months but there was no improvement on conservative management. During the eighth week cortisone therapy was started in doses of 100 mg. per day. An elevated serum bilirubin and alkaline phosphatase developed. The bilirubinemia cleared within one week but the elevated alkaline phosphatase persisted throughout the three and one-half months prior to the patient's death.

The postmortem examination confirmed the diagnosis of systemic lupus. The additional diagnosis of miliary tuberculosis of lungs, spleen, liver and kidney was established. The common bile duct was found to be patent. The gallbladder had been removed surgically twenty years before her admission.

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1952					
Nov. 3...	....	1.1	0.8	2+	7.5
Nov. 25...	49.7	1.0	0.8	2+	6.8
Dec. 10...	30.2	...	...	...	...
1953					
Jan. 2....	27.9	...	...	...	...
Jan. 12...	25.5	...	...	...	...
Jan. 23...	30.3	...	...	...	...
Feb. 3....	38.5	...	...	...	...
Feb. 11...	39.7	0.8	...	2+	6.5
Feb. 12...	42.0	0.8	...	...	...
Feb. 19...	35.7	...	...	...	...

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1951					
April 24...	52.	0.8	...	3+	3.0
May 22...	....	0.8	...	...	...
June 4....	80.6	5.0	4.0	0	1.8
June 5....	75.1	...	...	...	...
June 7....	81.9	3.5	2.7	...	...
June 8....	70.1	2.4	1.6	...	...
June 9....	70.3	1.5	1.2	...	...
June 11...	53.4	1.2	0.8	...	...
June 12...	58.1	...	...	...	...
June 13...	50.4	1.4	1.0	...	...
June 14...	75.7	...	...	...	...
June 15...	53.9	1.1	0.8	...	...
June 18...	25.2	1.1	0.8	...	...
June 23...	40.0	0.8	...	...	...
June 27...	20.9	0.8	...	...	...

CASE V. F. G. (J. H. H. No. 510900), a forty-nine year old Negro farmer, died in 1952 three years after the diagnosis of Hodgkin's disease was established by a biopsy specimen of a lymph node. The illness was characterized by weight loss, adenopathy, weakness and pancytopenia. The liver increased in size as the disease progressed. Saber shins and scoliosis had been present since infancy. This process was thought to be either "rachitic or congenital." Complete roentgenographic survey of the bones revealed no evidence of active disease of the bone.

At postmortem examination typical infiltrations of Hodgkin's disease were seen in spleen, liver, kidney and bone marrow.

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1949					
Aug. 2....	31.0	1.0	0.8	0	1.6
Aug. 29...	32.9	0.8	...	0	2.5
Nov. 26...	20.9	1.6	0.8	0	0.8
1950					
Feb. 9....	34.4	0.8	0.8	3+	8.0
1951					
Nov. 14...	47.7	0.8	...	0	1.4
1952					
Feb. 8....	72.9	1.2	0.8	0	0.6
Feb. 25...	56.9	...	...	...	...
Mar. 27...	43.6	1.4	0.8	2+	1.2
June 2....	98.5	7.4	5.4	2+	0.6

CASE VI. O. C. (J. H. H. No. 655032), a twenty-nine year old Negro woman, was first admitted in October, 1953, complaining of fever and joint pains. The diagnosis of systemic lupus was confirmed by the finding of L.E. cells in the peripheral blood. After two months of cortisone therapy in the hospital the patient signed out against advice. She returned one year later with fever, debilitation and active joint disease. While on cortisone therapy massive gastrointestinal bleeding developed and the patient died in approximately one month.

At autopsy the liver weighed 1,800 gm. The extrahepatic ducts were all patent and of normal size. Sections of the liver showed central lobular congestion and atrophy. There were occasional areas of focal necrosis where slight hydropic changes were noted in the cells.

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity
1953					
Oct. 21...	4.3	...	...	2+	9.8
Nov. 12..	....	0.8	0	3+	7.2
Dec. 11...	6.0	0.8	0	3+	8.0
Dec. 18...	5.3	0.8	0	2+	4.4
1954					
Oct. 4....	41.2	0.8	0	3+	15.0
Oct. 8....	25.2	...	...	...	....
Oct. 29...	6.1	...	...	...	....
Nov. 1....	17.8	0.8	0.8	0	1.8

CASE VII. S. G. (J. H. H. No. 609324), a twenty year old Negro woman, was first seen in 1950 at which time x-ray of the chest showed haziness of the left base and an infiltrate in the right upper lobe. In 1952 this patient was admitted with cough, dyspnea, weight loss, fever, polyarthritis, hepatomegaly and confusion. The serum alkaline phosphatase was found to be elevated for the first time on this admission. During the period from 1952 to 1954 the patient had attacks of polyarthritis, pleurisy, evanescent lung infiltrates and subcutaneous nodules. She was admitted in 1954 with erythema nodosum. The liver and spleen were both enlarged. In addition to the typical lesions of erythema nodosum the patient had swelling and tenderness of both knees. Laboratory examination revealed a microcytic hypochromic anemia, white cell count of 10,000 to 16,000 per cu. mm. microscopic hematuria, serum globulin of 3.7 gm. per cent and albumin of 2.9 gm. per cent.

At the time of discharge the diagnosis was collagen vascular disease, probably systemic lupus erythematosus. The patient was treated with cortisone to which she responded dramatically.

Date	Alkaline Phosphatase (Bodansky units)	Total Bilirubin (mg. per cent)	Direct Bilirubin (mg. per cent)	Cephalin Flocculation	Thymol Turbidity	Bromsulphalein
1952						
June 16..	27.7	0.8	...	0	1.5	...
June 19..	24.3	...	...	..	...	...
1953						
July 21..	22.7	...	...	0	1.3	...
1954						
Oct. 28..	....	0.8	...	..	...	...
Nov. 17.	36.6	1.1	0.8	0	2.5	...
Nov. 30.	28.4	...	...	0	2.0	...
Dec. 3...	....	0.8	...	..	...	...
Dec. 11..	27.9	0.8	...	0	1.4	1.4

The finding of an elevated serum alkaline phosphatase activity in the presence of a relatively normal bilirubin in situations in which metastatic carcinoma and Paget's disease can be excluded may be of help to the clinician in differential diagnosis. This pattern is occasionally seen in patients undergoing diagnostic study because of fever, weight loss and hepatomegaly, and in some instances may provide an indication for a biopsy of the liver. In this connection it should be reiterated that chronic intermittent extrahepatic biliary obstruction in the remittent

phase can present an identical pattern. It is impossible to distinguish intra- from extrahepatic biliary obstruction by the routine biochemical examinations in general use. This point is well illustrated by the following case.

W. T. (J. H. H. No. 640815), a sixty-four year old white man, was first seen in May, 1953, at which time he complained of intermittent attacks of chills and fever of ten months' duration associated with a 20 pound weight loss. The attacks came at irregular intervals but as often as two per week. The first symptom was general malaise, followed by a chill and fever to 104 to 105°F. There was profuse sweating and defervescence within eight hours but the patient felt tired and weak for several days.

A slightly enlarged liver was noted on physical examination but no other abnormalities were detected. Blood chemical examination at this time revealed an alkaline phosphatase of 50.6 Bodansky units and a bilirubin of 2.7 mg. per cent total and 1.8 direct. The presumptive diagnosis was abdominal lymphoma or tuberculosis and an exploratory laparotomy was performed. No abnormal masses were found; a biopsy specimen of the liver was essentially normal; and the gallbladder and bile ducts were normal to inspection. The common bile duct was not opened. The patient was discharged with fever of unknown origin as the only diagnosis.

During the next six months he continued to have the attacks of chills and fever. The striking elevation of serum alkaline phosphatase activity persisted, with values ranging from 25 to 70.2 Bodansky units. The total bilirubin ranged from 1.5 to 3.0 mg. per cent. At no time did the patient complain of abdominal pain.

At this time the diagnosis of Charcot's intermittent biliary fever was suspected and a rise in bilirubin from 1.6 to 3.0 mg. per cent was documented during an attack of fever. The patient was re-explored and a large stone was found in the common bile duct. A probe could not be passed into the duodenum through the common bile duct until the duodenum was opened and the sphincter of Oddi incised. The gallbladder was removed.

The patient was well for approximately one year after operation when the attacks of chills, fever and jaundice returned. The patient was explored a third time and a large amount of biliary sludge was removed from the common duct and a T-tube left in place. Three months after operation the tube was draining well and the patient was asymptomatic.

This case serves to emphasize that obstruction of the biliary tract should be suspected in all patients with an elevated serum alkaline phosphatase activity and that the gallbladder and common duct may appear normal at operation in the presence of biliary obstruction. Biliary obstruction due to gall stones can be cured and therefore must be the first consideration when the serum alkaline phosphatase activity is elevated. It is our belief that all patients with unexplained elevation of serum alkaline phosphatase activity should be subjected to abdominal laparotomy and the common duct thoroughly explored.

COMMENTS

The alkaline phosphatases are globulins of low molecular weight. They are found in every tissue of the body and are present in bone, liver, kidney and intestinal mucosa in high concentration.[13] Alkaline phosphatase appears in the blood, thoracic duct lymph, bile and feces, but not in the urine.[14,15] Many efforts have been made to identify the tissue of origin of the phosphatase in the serum by the chemical characteristics of the enzyme.[16,17] The results of this approach have been inconclusive.

There are two theories concerning the elevation of serum alkaline phosphatase activity in disease of the liver and biliary tract. The retention theory proposes that the serum enzyme has its origin largely in bone and is actively secreted by the liver cells into the bile and thus eliminated from the body. The evidence for this theory is based upon the known occurrence of phosphatase in bone, the elevation of serum alkaline phosphatase activity in disease of the bone and the appearance of phosphatase in the bile. The infusion of phosphatase is followed by an increased excretion of phosphatase in the bile.[18] Any compromise to the patency of the biliary ducts interferes with the excretion and leads to retention of the phosphatase in the serum. In general, this explanation correlates so well with clinical experience that the retention theory has gained wide support.

The second or hepatogenic theory proposes that phosphatase is produced largely by the liver and in the presence of biliary obstruction is released into the blood stream. According to the hepatogenic theory, two conditions seem essential for the elevation of serum alkaline phosphatase activity in disease of the liver: that at least part of the liver be capable of producing alkaline phosphatase and that the biliary drainage from that portion of the liver be obstructed. The hepatogenic theory is supported by numerous clinical and experimental observations.[19–23] It has been suggested that conditions producing intermittent or partial biliary obstruction or

inflammation of the smaller biliary radicals seem to enhance the production of phosphatase by the liver and are associated with high serum levels.[24–26] Prolonged complete obstruction, on the other hand, is characterized by an eventual decrease in the phosphatase level suggesting that the liver's ability to produce the enzyme has been impaired.[25,27] The serum alkaline phosphatase levels following ligation of the common bile duct in the dog are higher than those following total hepatectomy.[23] Although ligation of one of the branches of the hepatic duct produces a marked rise in serum alkaline phosphatase activity (and no change in the serum bilirubin),[28] this same ligation combined with ablation of the portion of the liver drained by the ligated duct produces almost no change in the serum phosphatase.[29] Severe necrotizing hepatitis (due to a virus or a chemical agent) does not usually produce a marked rise in the serum alkaline phosphatase.[20] These observations indicate that the liver may produce as well as excrete the serum enzyme.

Thus both the liver and bone can produce alkaline phosphatase and the source of the increased serum alkaline phosphatase in disease of the liver remains a subject of controversy. Enzyme inhibitors have been employed to evaluate the contribution of liver and bone to the elevated serum alkaline phosphatase activity and such studies on bloods with elevated serum alkaline phosphatase activity due to disease of the liver have yielded conflicting results.[30,31] After hepatectomy the serum alkaline phosphatase activity neither falls to zero nor rises as rapidly as following ligation of the common bile duct. It is apparent that neither theory alone can explain this phenomenon. The retention theory fits most clinical experience, but in some situations the hepatogenic theory offers a better explanation for the available facts.

The paper of Gutman et al.[3] on the quantitative relationship of serum alkaline phosphatase activity and disease of the biliary tract summarizes current thought in this sphere. They state: "With certain unexplained exceptions, the level of phosphatase activity of the serum in the adult appears to be peculiarly sensitive to any significant compromise of the patency of the extra or intrahepatic biliary system, but is relatively unaffected by even extensive liver parenchymal injury per se." Gutman also calls attention to situations in which the phosphatase and bilirubin do not rise proportionately. "Jaundice is a late and inconstant manifestation of metastatic malignancy . . . usually before there is any demonstrable liver enlargement, the serum phosphatase activity of patients with liver metastasis often shows a distinct rise. . . . "

The findings in patients with tuberculosis, sarcoid, Hodgkin's disease and amyloidosis reported here are similar to those in patients with metastatic malignancy. Infiltration by granulomatous or malignant tissue is presumed to be the common denominator in all these situations. An identical chemical pattern in the serum may develop when fibrous tissue contracts, as in biliary cirrhosis or the cirrhosis of arsenical jaundice or ulcerative colitis.

It is difficult to attribute the elevation of serum alkaline phosphatase activity in the patients with infiltrative disease of the liver reported herein to intrahepatic biliary obstruction in that no involvement of the portal areas was seen in two patients (Cases III and VI). In fact, microscopic changes in the liver were predominantly in the central areas and were considered to be of minimal degree. Obstruction of the biliary drainage from even a small part of the liver can result in an elevation of serum alkaline phosphatase activity, and it is possible that such areas were overlooked in these cases or that the disease process interferes with bile pigment metabolism and excretion in ways not revealed by routine microscopic examination. An alternative explanation is that the elevation of serum alkaline phosphatase activity may be related to overproduction of the enzyme rather than to decreased excretion. The alteration in serum globulin observed in many of the diseases under discussion suggests that the elevation of serum alkaline phosphatase activity might be related to protein synthesis. The pathogenesis of the elevation of serum alkaline phosphatase activity in this group of patients remains obscure but on the basis of the information at hand cannot be attributed to intrahepatic biliary obstruction alone.

SUMMARY

The diagnostic significance of an elevation of serum alkaline phosphatase in the presence of a normal serum bilirubin is discussed. Attention is called to a group of patients with chronic infiltrative disease of the liver due to sarcoid, tuberculosis, Hodgkin's disease and other similar conditions in which this combination exists. Although less frequent than biliary obstruction,

Paget's disease and metastatic carcinoma, these cases may present as diagnostic problems and knowledge of this chemical pattern may be helpful. Obstruction of the biliary duct should always be considered first when the serum alkaline phosphatase is found to be elevated as the therapeutic possibilities are brighter than in the case of any of the diseases under consideration herein.

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Hemochromatosis and Hemosiderosis*

Does Iron Overload Cause Diffuse Fibrosis of the Liver?

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"Wie will man denn mit Sicherheit entscheiden, welches von zwein neben einander existirenden Dingen Ursache und welches Wirkung sei, und ob überhaupt eines von veiden Ursache und nicht vielmehr beide Coeffecte derselben dritten Ursache, oder gar jedes für sich Effect zweier ganz verschiedener Ursachen sei?"

How then can one with certainty determine which of two concurrent phenomena is cause and which effect, and whether either is in fact cause and both are not simultaneous effects of a third factor or, indeed, that each is not the effect of two quite distinct causes?

VIRCHOW, 1847

ALTHOUGH generally discarded two decades ago, largely due to the influence of Sheldon's monograph[1] the idea that hemochromatosis is due to the effects of excessive accumulation of iron in the tissues has gained many new supporters in recent years.[2–6] This change in the climate of opinion, which has led many clinicians to equate hemochromatosis with hemosiderosis, has been brought about to some extent by presentation of new clinical data and better understanding of iron metabolism, but is partly a result of confusion of terminology and uncritical examination of the evidence. In fact much has been written in apparent ignorance of a large body of well established clinical and experimental evidence. None of the experimental work on animals presented in recent years contradicts the findings of Rous and Oliver,[7] Polson[8] and Cappell,[9] all of whom were unable to reproduce the histologic picture of hemochromatosis in the livers of rats and rabbits by the administration of iron compounds. Diffuse fibrosis of the liver with distortion of the lobular pattern, similar to the picture seen in Laennec's cirrhosis, is the single constant feature of hemochromatosis.

The debate should be centered about the question of whether or not excessive iron pigment deposition leads to fibrosis of the liver directly or indirectly. Granick,[4] to whom we are indebted for some of the newer knowledge of iron transport mechanisms, expressed a conservative point of view when he admitted that it had not been demonstrated that the accumulation of iron in the tissues as ferritin or hemosiderin brought about cell destruction and fibrosis. He believed it was more reasonable to suppose that iron which was not converted into the proper storage compound might cause cell destruction by precipitating proteins indiscriminately or, perhaps, by inactivating enzymes in the liver so as to interfere with its resistance to various noxae. Finch and his co-workers[5] were less cautious. They stated that regardless of how the iron initially arrived in the tissues, a redistribution of it later occurred and the liver became progressively overloaded. Following this overloading the secondary receptors began to fill. Fibrosis then occurred in the organs most heavily laden with iron. After stating that this sequence of events was responsible for the clinical and pathologic picture of hemochromatosis Finch et al. retreated from this rather extreme position; they noted that the question of the extent to which iron pigment was responsible for fibrosis of the liver was still open since no great success had been achieved in the production of such a lesion in experimental animals by means of iron injections or blood administration. To these investigators the clinical occurrence fibrosis of the liver after multiple transfusions seemed fairly convincing evidence in favor of their view.

That no great success has been achieved in producing fibrosis of the liver in experimental animals by the administration of iron is an understatement. No success at all has been achieved and numerous attempts have been made. Of the recent workers Kinney et al.[10] and Wyatt and Howell[11] were unable to produce the lesion. Various objections to these experi-

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TABLE I

Rat No.	Diet (no. of days)	Body Weight at Death (gm.)	Liver Weight at Death (gm.)	Age (in days)	Manner of Death	Iron in Liver (mg./100 gm. dry wt.)
DIET I						
102825	180	425	16.1	270	Killed	37
102826	180	360	11.1	270	Killed	38
102827	180	390	11.0	270	Killed	30
102828	180	325	11.2	270	Killed	40
102829	180	360	11.7	270	Killed	44
102830	180	365	10.3	270	Killed	52
102831	180	420	12.2	270	Killed	38
102832	180	410	11.7	270	Killed	32
102833	180	405	14.3	270	Killed	41
102834	180	410	12.1	270	Killed	32
DIET II						
97415	485	325	12.4	475	Killed	....
97414	385	345	14.9	475	Killed	1020
97410	385	348	11.1	475	Killed	810
97411	385	278	11.4	475	Killed	1400
97420	386	420	15.8	476	Killed	720
97424	386	280	13.2	476	Killed	1070
97423	386	350	11.3	476	Killed	940
97422	386	350	16.9	476	Killed	1050
97426	386	285	14.7	476	Killed	890
97412	351	250	15.0	441	Died	1250
97418	244	270	19.1	334	Died	1550
97416	214	265	18.4	355	Died	880
97425	288	220	9.7	378	Died	1110
97419	363	438	23.7	453	Died	890
DIET III						
96483	400	220	10.7	490	Died	104
96481	379	258	6.7	469	Killed	111
96443	518	500	15.9	608	Killed	52
96439	521	485	11.1	611	Killed	49
96467–1	521	470	17.5	611	Killed	90
96440	521	500	15.5	611	Killed	58
96494	521	488	16.5	611	Killed	90
96400	521	460	16.7	611	Killed	41
96482	521	470	12.1	611	Killed	68
96441	271	370	9.8	361	Died	....
DIET IV						
96478	486	145	10.3	576	Died	1750
96451	109	143	7.4	199	Died	....
96453	283	200	8.8	373	Died	1210
96457	162	328	23.0	251	Died	....
96465	485	170	9.0	575	Died	2070
96454	133	125	8.7	223	Died	....
96467	442	205	12.4	532	Died	3180
96469	272	155	9.8	363	Died	1095
96479	518	245	10.3	608	Killed	2400
96452	518	383	15.6	608	Killed	1845
96466	518	320	18.9	608	Killed	1200
96456	255	163	8.7	345	Died	....

Arithmetical Averages: Diet I (all animals) 38.5 mg. Diet II (all animals) 1,070; (animals killed) 1,005. Diet III (all animals) 73.88. Diet IV (all animals) 1,775; (animals killed) 1,815.

ments have been raised. It has been said that the concentration of iron reached in the liver was not high enough, that in these experiments the time allowed for the development of fibrosis was insufficient and, finally, that the animals chosen for the experiments were unsuitable. These objections have some weight, although the last, in the absence of any evidence to support it, is more of a delaying action than an argument. The experiments of Wyatt and Howell and of Kinney et al. on rats were only a few months in duration and the concentrations of iron attained in the livers were not very high. On the other hand Polson[8] treated rabbits with intravenous colloidal iron over a period of three years or more. The livers of these animals contained from 3,420 to 7,670 mg. (average 5,190 mg.) iron per 100 gm. of dry tissue, amounts somewhat higher than those reported in most cases of hemochromatosis. Nissim,[12] however, pointed out that most of the iron administered in colloidal form ends up in reticuloendothelial cells, represented in the liver by the Kupffer cells, and unless it can be shown that parenchymal cells of the liver contain amounts of iron pigment comparable with hemochromatosis, the question of the toxicity of iron remains unanswered. Nissim believed that he had succeeded in producing lesions in the liver and elsewhere with massive intravenous doses of iron salts but did not consider these lesions characteristic of hemochromatosis.

In the experiments reported here, ferric citrate was fed to rats on high and low protein diets over periods equivalent to one-half to two-thirds their normal life span. Stainable hemosiderin in the parenchymal cells of the liver and total iron in the whole liver tissue accumulated in amounts equivalent to those observed in hemochromatosis.

METHODS

Ninety day old male rats of the Addis colony at Stanford (derived from the Slonaker strain) were placed on diets of the following compositions:

Diet I (*Stock diet*): Soybean meal 36 parts, whole wheat 42 parts, cornstarch 12 parts, bone ash 0.9 parts, alfalfa 0.8 parts, salt 0.3 parts, sardine oil 1.9 parts. Approximately 16 per cent protein.

Diet II: Stock diet plus 6 per cent ferric citrate.

Diet III (*low protein diet*): Soybean meal 20 parts, whole wheat 20 parts, cornstarch 56 parts, bone ash 0.9 parts, alfalfa 0.8 parts, salt 0.3 parts, sardine oil 1.9 parts. Approximately 4 per cent protein.

Diet IV: Low protein diet plus 6 per cent ferric citrate.

The rats were killed or died of intercurrent disease (usually pneumonia) as indicated in Table I. The organs were fixed in formalin and subsequently embedded in paraffin. Iron was demonstrated by treating tissue sections for thirty minutes at room temperature

with freshly mixed 2 per cent hydrochloric acid and 2 per cent potassium ferrocyanide in equal proportions. Spectrographic analysis of the iron content of the livers was made using a technic described elsewhere.[13] Spectrographic analysis of the diets showed 30 mg. iron per 100 gm. of Diet I, 38.1 mg. iron per 100 gm. of Diet III and 1,080 mg. per 100 gm. of Diet IV. At the start of the experiment 2 per cent ferric citrate was added to the stock and low protein diets. Biopsy specimens of the liver taken one month after the experiment began showed an unsatisfactory accumulation of iron, therefore, the concentration of ferric citrate was raised to 6 per cent in both diets.

RESULTS

The quantitative data are shown in Table I.

Gross Changes. The livers of the rats on Diets II and IV were reddish brown; this appearance was more striking in the rats on Diet IV. The livers in two of the rats on this diet had wrinkled surfaces, an appearance previously described by Nissim.[12] In one rat (No. 96481), of the low protein control group (Diet III), the liver was grossly nodular and cut with slightly increased resistance.

Histologic Findings. Liver: In all the rats on diets containing ferric citrate there was a variable but always large amount of golden brown pigment in the parenchymal and Kupffer cells. All this pigment turned deep blue after treatment of the sections with hydrochloric acid and potassium ferrocyanide. The ratio of the amount of pigment found in the parenchymal and Kupffer cells differed from rat to rat but in only one or two instances did the pigment seem to be predominantly in the parenchymal cells. In many instances the Kupffer cells were clustered together in rounded nodules. These nodules were not associated with local fibrosis. The hemosiderin in the parenchymal cells appeared in the form of fine, discrete particles which avoided the sinusoidal margins but extended to the margins of the adjacent cells so that a filigreed pattern was produced. (Fig. 1.) The deposition of pigment in the periportal zones exceeded that in the pericentral zones. No iron-free pigment was present. Diffuse fibrosis of the liver did not occur in any of the rats fed ferric citrate nor did it occur in the rats fed the stock diet. The liver of one rat (No. 96481) in the low protein control group, however, showed mild fibrosis, chiefly centrolobular. Bands of collagenous tissue occasionally linked several lobules together. Centrolobular atrophy and necrosis were present. The lobular pattern was not distorted. There was more iron in the liver of this rat than in the liver of any of the other control fats and hemosiderin-containing macrophages were scattered throughout. Hemosiderin was not present in the parenchymal cells.

Pancreas: Only in one rat (No. 96465, Diet IV) were there more than a few iron-positive granules in the acinar cells. No iron-positive granules were seen in the islets. Fibrosis did not occur.

Abdominal lymph nodes: In the rats on Diets II and IV the abdominal lymph nodes were packed with hemosiderin. Judging from visual inspection there was more hemosiderin in these nodes than in either the liver or spleen.

Spleen: Large amounts of iron were present in the red pulp of all rats on Diets II and IV.

Kidneys: Both proximal and distal tubules, insofar as these segments could be accurately localized, contained varicolored droplets in the sections stained for iron. A few of the droplets were deep blue, most were a lighter blue, some were blue-green, light green or golden. They varied in size from fine dust-like particles (usually deep blue) to larger "hyaline droplets" from two to four microns in diameter.

Adrenals: A few fine blue particles and a diffuse bluish staining of the cytoplasm were observed in the glomerulosa zones of the rats on Diet IV. In a few of the rats on Diet II approximately the same amount of iron was present while the other rats had lesser quantities dwindling down to complete absence.

Reticuloendothelial system: Pigment-bearing cells interpreted as phagocytes were scattered through the adrenal medulla, bone marrow, interstitial tissue of the heart and elsewhere. They were not numerous if those in the liver and spleen are excluded from the reckoning. The bone marrow in particular contained very little pigment.

Skin, hypophysis, thyroid and salivary glands: Iron-containing pigment was not observed in these tissues.

Testes: All the rats on the ferric citrate diets showed severe testicular atrophy. This was not observed in either control group.

COMMENTS

Prior to a discussion of the main point at issue, there are several experimental findings which require comment. The testicular atrophy present in all the rats on the diets containing ferric citrate was most probably due to nutri-

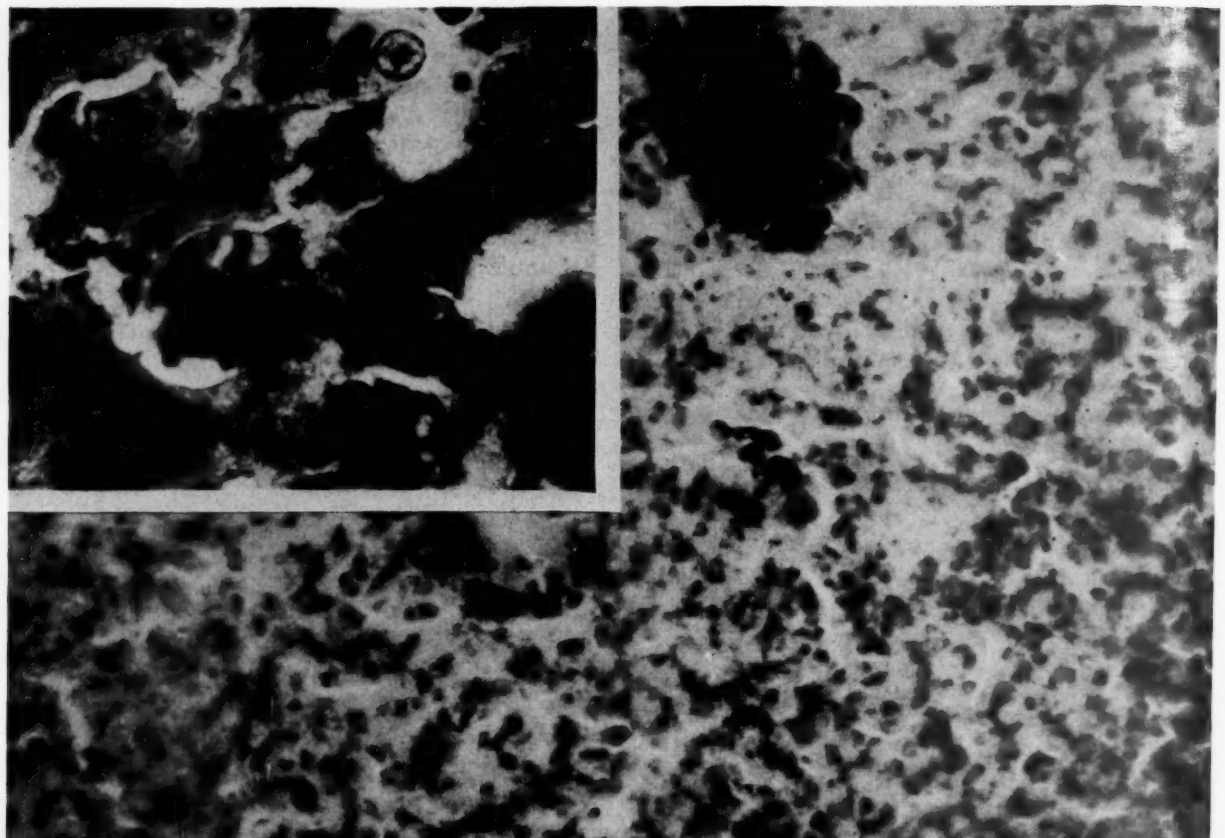

FIG. 1. Low protein-ferric citrate (rat No. 96456, Diet IV). Prussian blue reaction. All the dark granules and clumps are positively reacting material (hemosiderin) and deep blue on the slide. Note the filigreed pattern of the finely granular particles of hemosiderin in the parenchymal cells and the coarse clumps in the Kupffer cells. The latter form large aggregates. Original magnification, × 150. Insert: same preparation, original magnification, × 600. Note how the hemosiderin accumulates in the central portion of the parenchymal cell cytoplasm, leaving the lateral margins free. The roughly rounded dark mass in the left lower quadrant of the insert represents a Kupffer cell bulging into a sinusoid.

tional deficiency, although some of the animals on the standard protein diet plus ferric citrate (Diet II) appeared to be in good physical condition. Still, the relative infrequency of testicular atrophy in inactive or "healed" cirrhosis,[14] the aforementioned experimental finding and the fact that although the liver lesion in classic hemochromatosis falls into the category of inactive cirrhosis, testicular atrophy is almost invariably present, introduce the possibility that excess iron may interfere with the proliferation and differentiation of germinal epithelium. The experimental results show that the iron barrier can be overcome in rats when a sufficiently high concentration of iron is given in an otherwise normal diet. Whether this is true for other species is not known although it seems likely. The pattern of distribution of hemosiderin in the tissues of the iron-fed rats, aside from pigmentation of the adrenal glomerulosa, bears relatively little resemblance to that seen in hemochromatosis since the other endocrine glands and the pancreas are spared while the reticuloendothelial system is heavily involved.

Although it is well known to students of disease that "classic" hemochromatosis occurs for the most part in persons who are not anemic and who have not received repeated transfusions or large

quantities of iron, some believe that the circumstances permitting the entry of excess iron into the tissues have no bearing on the question. The crux of the matter is thought by this group to lie in the damaging effects which excess iron exerts on the tissues regardless of its portal of entry. The question of this identity of advanced hemosiderosis and hemochromatosis cannot be settled without preliminary agreement as to criteria of "sameness." It is useless for the proponent of the view that hemochromatosis and hemosiderosis are different entities to insist on differences believed due to the arbitrary grouping of cases by his opponent who holds that hemochromatosis and hemosiderosis are the same entity. The latter, apparently, believes that the classic picture of hemochromatosis was created by arbitrarily selecting those cases of advanced hemosiderosis of unknown origin which happen to present cirrhosis, diabetes and a particular type of pigment distribution in the skin and viscera.

There are distinctions between the tissue patterns of pigment deposition in classic hemochromatosis and in hemosiderosis. These distinctions, as well as the presence of an acid-fast, iron-free pigment, named hemofuscin by von Recklinghausen, in many of the classic instances of hemochromatosis have been stressed by pathologists interested in the problem of differentiating hemosiderosis and hemochromatosis. However, it is possible to hold that these distinctions have no bearing on the question of the essential identity of the two conditions; as little, say, as the question of the localization of tubercles would have in settling the problem of the etiologic identity of pulmonary tuberculosis and lupus vulgaris. The question of the significance of hemofuscin has been complicated by a lack of agreement as to its nature and seemingly great quantitative variations in its occurrence. Pearse[15] includes hemofuscin with the lipofuscins, a group of pigments derived from lipid or lipoprotein sources with many subdivisions characterized by differences in reactivity to various tests (acid-fastness, PAS reaction, fluorescence, Schmorl's reaction, basophilia, Sudan stains and silver reduction). Only arbitrary distinctions can be made among the members of this group of compounds. The reactions of these compounds to the various tests is thought to depend chiefly on their state of oxidation. The question is further complicated by the recent demonstration of Gedigk and Strauss[16] of the somewhat similar tinctorial and fluorescent properties shared by hemosiderin in tissue sections from which the iron has been removed by incubation in hydrochloric acid. According to Pearse certain acid fixatives act on hemosiderin to remove the iron, or render it inactive toward potassium ferrocyanide and hydrochloric acid, yielding a compound, aposiderin, which has the characteristics of lipochromes of medium and high grades of oxidation. It does not seem, all things considered, that the presence of hemofuscin is pathognomonic of hemochromatosis.

Putting these distinctions aside, then, we are left with the central problem of whether or not the presence of excess iron-containing pigment in the liver causes diffuse fibrosis. If this question is answered in the affirmative, hemochromatosis may justifiably be considered a special instance of hemosiderosis. However, it is just this point on which the evidence is weakest. Sheldon[1] reviewed the experimental and clinical evidence up to the middle thirties and came to the conclusion that a damaging effect of iron pigment on the tissues was unproved and seemed unlikely since there was no correction between pigment deposition and degree of fibrosis. As previously stated, none of the recent experimental evidence has contradicted the earlier work. The well established fact that pneumoconiotic siderosis is not associated with fibrosis of the lungs has been cited by Dubin[17] as a piece of negative evidence, but in this condition the iron is not deposited in the tissues in the form of hemosiderin. Only when there is long-standing chronic passive congestion of the lungs are hemosiderin and fibrosis sometimes associated. Pulmonary fibrosis also occurs, however, in chronic passive congestion without deposition of hemosiderin. The clinical evidence cited in support of the affirmative position, that is the fact that fibrosis of the liver has been found at autopsy in some cases of refractory anemia with multiple transfusions obviously does not establish the existence or sequence of a relationship between the two. Several possibilities present themselves. Some of these patients may have acquired viral hepatitis in the course of multiple transfusions. It is quite possible that other patients may have had hemochromatosis with anemia of the megaloblastic type, recently reported on by Koszewski,[18] before the transfusions were given. One must consider that: (1) fibrosis of the liver, characteristic of Laennec's cirrhosis, could precede the hemosiderosis, (2) hemosiderosis of the liver could precede and cause fibrosis (Finch's view), (3) the fibrosis

and hemosiderosis of the liver might not be causally related except through a third factor, and (4) the occurrence of the two together might be fortuitous.

Some confusion is introduced by our nomenclature which fails to distinguish between diseases and lesions. "Laennec's cirrhosis" designates a disease with certain anatomic and metabolic abnormalities (among which is one which may lead to the increased uptake of iron and its accumulation as hemosiderin in the liver and occasionally in other tissues). Fibrosis of the liver with or without distortion of the lobular pattern is completely characterizable from an anatomic standpoint. Thus, from the point of view held by Finch, hemosiderosis is a cause of diffuse fibrosis of the liver; the proliferated fibrous tissue in this context being regarded not as evidence of a disease with a long life history and a complex of metabolic and other abnormalities but simply as an anatomic lesion.

Hedinger[19] and Herbut[20] have presented evidence supporting a relationship between Laennec's cirrhosis and hemochromatosis. Dubin also emphasized the similarities between these two diseases but believed that they were basically different entities. The evidence may be summarized as follows: (1) the morphologic changes in the liver, except for the differences in iron content, are similar; (2) in those instances of Laennec's cirrhosis in which the presence of iron pigment was sought, it was found in from one-third to one-half of the cases and a special category of "pigmented cirrhosis" has been proposed to accomodate these cases; (3) fibrosis of the pancreas is frequently found in Laennec's cirrhosis; Herbut observed it in fifty-one of sixty cases in which histologic sections were available for reexamination; (4) diabetes is not uncommon in Laennec's cirrhosis; Herbut reported twelve cases of diabetes in one hundred and fifteen necropsied cases of cirrhosis and found in the literature one hundred twenty-one reports of non-pigmentary cirrhosis associated with diabetes; (5) testicular atrophy occurs commonly in both conditions;[1,14] and (6) increased melanin pigmentation of the skin is common in both conditions.[21]

Sheldon considered some of these arguments and, while admitting their strength, rejected the conclusion because of: (1) the sex incidence of twenty to one in favor of males in his series of over 400 cases, (2) the more widespread deposition of iron pigment in the glandular organs, muscles, cartilage and synovial tissues in many cases of hemochromatosis and (3) the presence of hemofuscin in hemochromatosis. Sheldon also emphasized the quantitative dissimilarity and discontinuity of the iron content of the liver in the two diseases stating that cases with intermediate amounts of iron ought to be found on the hypothesis that they were variants of the same condition. Another argument which might have been presented is the presence of Mallory bodies (alcoholic hyalin) in active Laennec's cirrhosis and the absence of these inclusions as well as of the other criteria for activity[22] in almost all cases of hemochromatosis. The much higher incidence of alcoholism in Laennec's cirrhosis and the familial occurrence of hemochromatosis, which has been reported several times,[23] are also distinguishing features.

Owing to the periodic loss of menstrual blood the human female cannot accumulate iron in the tissues as quickly as the male. Because of this the normal adult female loses about 300 mg. of iron a year. In addition an equally large amount of iron may be secreted in the milk during lactation. The prevalence of hemochromatosis in males may be explainable on this simple basis. The argument that widespread distribution of iron pigment in "typical" cases of hemochromatosis establishes a significant difference between hemochromatosis and certain cases of Laennec's cirrhosis with iron pigmentation begs the question unless it is considered with the claim that transitional cases do not occur. In regard to this, a case in point was recently reported with the diagnosis of "hemochromatosis with megaloblastic anemia and cirrhosis with Mallory bodies."[24] A quantitative spectrographic measurement revealed 396 mg. iron per 100 gm. of dried liver, much less than that in any of the cases of hemochromatosis reported by Sheldon (1,040 to 7,620 mg. with an average of 3,650 mg.) and about seven or eight times the normal iron content of the liver. The anterior hypophysis, pancreas and thyroid were heavily pigmented in this case, the adrenal glomerulosa less so, while the hepatic lesion had all the characteristics of active Laennec's cirrhosis, that is, partially disintegrated liver cells, Mallory bodies (occurring in partially disintegrated cells or in cells with large nuclei and prominent nucleoli), proliferated bile ducts, leukocytic infiltration, distortion of normal lobular relations and absence or limited amounts of those sharply defined nodules of well preserved liver

cells lying in dense fibroelastic tissue, characteristic of healed or "inactive" cirrhosis. Most cases of hemochromatosis, as already mentioned, do not show evidence of activity in this sense. Mallory did not find intracytoplasmic "alcoholic hyalin" in cases of hemochromatosis. This instance of hemochromatosis with megaloblastic anemia and cirrhosis with Mallory bodies is very instructive since it shows that intermediates between Laennec's cirrhosis and hemochromatosis do occur. Another case in point may be cited:

Autopsy 3E-394 (San Francisco Hospital, Stanford Service). A fifty-three year old white woman, known to be addicted to alcohol, was first seen two years prior to her death when a diagnosis of Laennec's cirrhosis was made. The patient did not return to the clinic until disturbed by abdominal pain. At this time she had edema, ascites and mild jaundice. Hemoglobin on admission was 13.5 gm. A few days after admission the patient died following hematemesis, which was shown at autopsy to have originated in ruptured esophageal varices. Five liters of ascitic fluid and a diffusely nodular liver weighing 1,180 gm. were found. Both the liver and pancreas had a fine golden brown color. Histologic studies showed somewhat less iron pigment in the liver than is usual in hemochromatosis, 450 mg. per 100 gm. of dry weight by spectrographic analysis; fibrosis of the pancreas and hypertrophy of the islets with moderately large quantities of iron in both acinar and islet cells; and small quantities of iron pigment in the heart, thyroid, adrenal glomeruli and anterior pituitary. Applying the criteria previously listed the cirrhosis was not "active."

Granting the existence of a close relationship between ordinary Laennec's cirrhosis and hemochromatosis we are left without an explanation for the great variations in the uptake of iron. These variations are at least partly due to differences in diet since it has been observed that in geographical areas where "hemochromatosis" is common, there is a high incidence of "pigmented cirrhosis." The familial occurrence of hemochromatosis suggests that an inherited metabolic abnormality is somehow involved in the pathogenesis of the condition but at present it does not seem possible to draw any conclusions beyond this. While it is probably true, then, that some instances of active Laennec's cirrhosis may have all the earmarks of hemochromatosis, few cases of hemochromatosis show the peculiar histologic and cytologic features of "active" cirrhosis. This being so, if the hepatic lesions of Laennec's cirrhosis and hemochromatosis are always on the same basis, these lesions must develop very slowly in hemochromatosis perhaps entering a quiescent phase (as far as the destructive process involving the liver cells is concerned) while the metabolic error leading to the increased uptake of iron continues in effect. The higher incidence of alcoholism in Laennec's cirrhosis and the familial tendency in hemochromatosis indicate that other predisposing and pathogenic factors are involved in the genesis of the latter disease.

Dubin suggests biopsy of the gastric mucosa and an intravenous iron toleration test as a means of distinguishing hemochromatosis from Laennec's cirrhosis. It is doubtful whether these tests would do more than separate severe from milder cases. Dubin believes that enteric iron absorption is increased in hemochromatosis but not in Laennec's cirrhosis with pigmentation. It is difficult to see how this could be true in those rare cases of Laennec's cirrhosis with generalized pigmentation since it seems obvious from the reported values of iron in the liver that the total body iron must be increased. In the absence of transfusions there must have been increased iron absorption at some time. The intravenous iron toleration test is said by Dubin, admittedly on the basis of a few observations, to show a greater relative tissue uptake of iron in hemochromatosis than in Laennec's cirrhosis. These results, if extended and confirmed to cover cases of Laennec's cirrhosis with generalized pigmentation, would be of interest and would serve to establish a distinction although probably only one of degree. Dubin states that the hemosiderin of Laennec's cirrhosis is predominantly in the Kupffer cells. This has not been the writer's experience or that of other authors.[20,25]

The suggestion that hemosiderosis and fibrosis of the liver are related through a third factor has been made by several authors. Even prior to the era of repeated transfusions, the occurrence of diffuse fibrosis of the liver in association with varying degrees of hemosiderin pigmentation was not known. Rössle[26] introduced the term "angiohematotoxic cirrhosis" for cases in which primary damage appeared to be directed at the vasculature rather than at the parenchyma in the form of a "sclerosing capillaritis." He included Banti's syndrome and "pigmented cirrhosis" (not hemochromatosis) under this heading. As support for his contention that diffuse fibrosis of the liver might be an expression of a disease characterized by damage of the reticu-

loendothelial system and the blood-forming organs, he pointed to the occasional association of diffuse fibrosis of the liver with polycythemia, congenital jaundice, pernicious anemia, Niemann-Pick's disease and Gaucher's disease. In a review of generalized hemosiderosis with fibrosis of the liver and pancreas in Cooley's anemia, Ellis et al.[27] stated that although hemosiderosis and fibrosis generally paralleled one another, definitive evidence of a causal relationship between the two was lacking and the fibrosis could not be closely correlated with the duration of the disease (one of the most severe instances of hepatic fibrosis was in a three year old patient). Ellis et al. noted that chronic anemia of long duration was a common factor in cases of Cooley's anemia with fibrosis and in certain other cases of so-called transfusion hemochromatosis.

The fortuitous occurrence of hemosiderosis and fibrosis of the liver, in the absence of a causal relationship between the two or a third factor, is a possibility which must be considered since the occurrence of both conditions can be shown to be fortuitous in some clinical instances. Whether or not a superimposed chronic viral hepatitis acquired in the course of multiple transfusions, accounts for any of the reported cases of "transfusion hemochromatosis" would be difficult to determine. There is no doubt that such fortuitous combinations occur under other circumstances, both experimental and clinical. All work with rabbits on the production of fibrosis of the liver by experimental means, for example, is suspect because of the high incidence of coccidiosis infection among these animals. In the experiments reported herein one instance of fibrosis of the liver in a rat fed the low protein diet without supplementary ferric citrate was noted. Had this animal by chance been in the group fed the low protein diet plus ferric citrate a fortuitous combination of hemosiderosis and fibrosis would have occurred, unless the supplementary iron protected the rat against fibrosis of the liver. The most striking instances of what seems to be a chance association of fibrosis and hemosiderosis are reported by Higginson, Gerritsen and Walker.[28] In a study of 296 autopsies performed on male and female Bantus these authors noted excessive hemosiderosis* in 80 per cent of the cases, regardless of the cause of death. Diffuse scarring of the liver occurred both with and without hemosiderosis. The Bantu diet consists chiefly of a porridge fermented in iron pots at an acid pH. It is estimated that as much as 100 to 200 mg. iron may be ingested each day, an amount roughly equivalent to that consumed by the rats in the present writer's experiments. Higginson found a large amount of iron in the liver and spleen and relatively little in the heart and pancreas. He stressed the differences in distribution of the pigment in the lesions in the Bantus and in hemochromatosis. The following table, adapted from Higginson's article, shows that the fibrosis and hemosiderosis occurred independently of each other. In the lesions of the Bantus and the rats fed ferric citrate, the amount of hemosiderin in the Kupffer cells almost invariably exceeded that in the parenchymal cells. In a case reported a few years ago by Wallerstein and Robbins,[29] the patient, who suffered from chronic hemolytic anemia, took enormous doses of iron orally over a period of many years. At autopsy hemosiderin was found in the liver chiefly within the Kupffer cells. There was mild fibrosis of the liver, the scars in the portal areas were unconnected and no distortion of the lobular pattern was present. The case was classified as "hemochromatosis" but does not belong in that category.

TABLE II
ASSOCIATION OF SEVERE HEMOSIDEROSIS AND FIBROSIS OF THE LIVER

Total No. Cases	Severe Hemosiderosis (severe fibrosis)	Severe Hemosiderosis (no fibrosis)	Severe Fibrosis (no hemosiderosis)
118 Females...	4	4	4
178 Males.....	13	7	3

When one searches for evidence to support the claim that excessive deposits of iron-containing

* The authors refer to the condition as "siderosis" but since they describe hemosiderin, that is, a brownish pigment containing iron which gives the Prussian blue reaction and since the term "siderosis" is already in use to describe lesions in which iron salts, unaccompanied by the carrier substance in hemosiderin, are laid down in the tissues (sideropneumoconiosis, siderosis bulbi, siderosis of the spleen), it would seem preferable to use the term "hemosiderosis." The carrier substance, presumably apoferritin, is not derived from hemoglobin. Under certain circumstances parenterally introduced iron may be developed into a pigment histologically and histochemically indistinguishable from hemosiderin, as the experiments of Gedigk and Strauss[30] with subcutaneously injected colloidal ferric hydroxide have shown.

TABLE III

COMPARISON OF DISEASES CHARACTERIZED BY LIVER FIBROSIS, DEPOSITION OF IRON PIGMENT, OR BOTH

Disease	Liver Lesion	Lesions Elsewhere than in the Liver	Pathogenesis of Disease
Hemochromatosis Laennec's Cirrhosis	Diffuse fibrosis, nodular regeneration and bile duct proliferation. Iron pigment increased in about 40% of cases diagnosed as Laennec's cirrhosis. A small number of cases of the latter contain iron in amount and location (parenchymal) comparable to hemochromatosis. Histologically active[22] cirrhosis not usually associated with great increases in liver iron, but see reference 24. Fibrosis of the liver does not follow but proceeds or develops along with iron deposition.	Diffuse fibrosis of the pancreas with heavy iron deposition in hemochromatosis. Fibrosis of the pancreas without iron pigment in many cases of Laennec's cirrhosis.[20] Iron pigment usually confined to the liver in the latter disease (so-called pigmented cirrhosis) but rarely found in endocrine glands, heart and pancreas (in which case the diagnosis apt to be changed to hemochromatosis after autopsy). Testicular atrophy in almost all cases of hemochromatosis. Testicular atrophy in Laennec's cirrhosis is much more common in the histologically "active" variety.[14]	Alcohol and dietary factors important in the genesis of Laennec's cirrhosis but less important in connection with hemochromatosis. Inherited factors are concerned in the genesis of the latter.[23] Unknown metabolic defect in both leading to increased uptake of iron.
Refractory Anemia	Diffuse fibrosis develops in an appreciable number of cases, mainly but not invariably after multiple transfusions. The relation between pigmentation and fibrosis is inconstant. Fibrosis in some instances due to chronic viral hepatitis?	Deposition of iron pigment in tissues other than the liver may resemble that seen in hemochromatosis except for the heavier deposits in the spleen.	Increased iron intake, absorption or both. Multiple transfusions.
Nutritional Hemosiderosis (Experimental and Clinical) Nutritional Cirrhosis	Diffuse fibrosis may occur in nutritional hemosiderosis but the fibrosis is not caused by the deposition of pigment. Proliferation of bile ducts insignificant in nutritional cirrhosis with or without hemosiderosis. Kupffer cells contain large quantities of pigment and often form aggregates (unlike hemochromatosis)	Fibrosis of the pancreas rare and unrelated to iron pigmentation.[28] Deposition of iron pigment in tissues other than the liver may resemble that seen in hemochroma tosis except for the heavier deposits in the spleen.	Increased iron intake, absorption or both. Faulty diet.

pigment are responsible for diffuse fibrosis of the liver and distortion of the lobular pattern characteristic of hemochromatosis, almost none is to be found; the sole support for this point of view melting away when cases of so-called "transfusional hemochromatosis" are subjected to critical review. All the experimental and clinical evidence points away from this claim, as a matter of fact, it points toward a more complicated pathogenetic interrelationship of several different conditions, which Table III attempts to outline.

SUMMARY

Experimental work was undertaken to ascertain the long-term histologic effects in rats of diets containing 16 per cent and 4 per cent protein, with and without the addition of 6 per cent ferric citrate. Interest was centered on the liver, since the equation of hemosiderosis with hemochromatosis rests on the supposition that hemosiderin, or one of its components, has a sclerotogenic effect on this organ. Quantitative spectrophotometric measurements of iron were made in the four diets and in the livers of forty rats. The 16 per cent protein diet contained 30 mg. iron per 100 grams; the 4 per cent protein diet, 38 mg. iron; the 4 per cent diet plus 6 per cent ferric citrate, 1,080 mg. iron. The arithmetic mean iron content of the livers of rats fed the 16 per cent protein diet 180 days was 38.5 mg./100 gm. of dry liver tissue, standard

error (1.95); of the livers of the rats fed 16 per cent protein plus 6 per cent ferric citrate 352 days, 1,070 mg. (58.2); of the livers of the rats fed 4 per cent protein 489 days, 73.8 mg. (8.0); of the livers of the rats fed 4 per cent protein plus 6 per cent ferric citrate 434 days, 1,775.5 mg. (231). Hemosiderin was found chiefly in the parenchymal and Kupffer cells of the liver, in the spleen and in the abdominal lymph nodes, lesser amounts were present in the pancreas, kidney, adrenal glomerulosa and in widely dispersed phagocytic cells. Fibrosis of neither liver nor pancreas occurred. One rat on the low protein diet without additional iron had a nodular liver with mild fibrosis and central atrophy at the time of autopsy.

The bearing of these observations on the relations between hemochromatosis, transfusion hemosiderosis, Laennec's cirrhosis and nutritional hemosiderosis is discussed.

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A Study of 356 Carcinoids of the Gastrointestinal Tract*

Report of Four New Cases of the Carcinoid Syndrome

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THIS paper reviews the subject of carcinoid tumors (argentaffinomas) of the gastrointestinal tract and summarizes autopsy and record material at seven Boston hospitals. Four new cases of the carcinoid syndrome are described; three are considered definite, one probable.

Carcinoid tumors have been the subject of such interest and investigation in the past that it is not surprising to learn of new features being described, for some questions previously raised have not been conclusively answered. The articles of Forbus[1] and Cooke[2] review the early history and point out some controversies which surrounded these tumors. Briefly, the chief areas of controversy have concerned their malignant properties, their nature and their cells of origin.

The term "carcinoid" was first proposed by Oberndorfer[3] in 1907 who stated that these tumors did not metastasize and emphasized their benign nature. Gosset and Masson[4] and Masson,[5] using silver impregnation technics, demonstrated the Kulchitsky cells of the crypts of Lieberkühn to be the cells of origin, as had first been noted by Bunting.[1] With the use of silver impregnations, Gosset and Masson pointed out the similarity of the tumor cells to the chromaffin tissue of paraganglia. Significantly, in the light of the newly recognized carcinoid syndrome, they regarded these tumors as of endocrine origin. In his review of the subject of carcinoids in 1928, Forbus[1] favored the endocrine theory of Gosset and Masson over others then debated. He reported six cases of his own. Unfortunately Forbus stated that the tumors were generally harmless and did not metastasize. During the next decade as cases accumulated in the literature, the view that carcinoids were benign gradually gave way to recognition of their potential malignancy. Interest then fell off somewhat until, in 1953, two Swiss workers, Isler and Hedinger,[6] reported three cases of carcinoid arising in the ileum, metastatic to the liver, and accompanied by chronic endocarditis of the right side of the heart, with pulmonic stenosis and tricuspid insufficiency. They were the first to express the belief that these findings constituted a definite syndrome.

In 1952 Biorck, Axen and Thorson[7] in Sweden reported a similar case but did not recognize the syndrome at that time. In 1954 Thorson and co-workers[8] reported three cases of their own and added thirteen more from the world literature including those of Isler and Hedinger. Thorson and co-workers noted the association of clinical features: flushing of the skin, an unusual type of cyanosis, frequent watery stools, bronchoconstrictive episodes resembling asthma, edema and ascites (the result of right-sided heart failure), all accompanied with abnormalities of the right side of the heart at autopsy, notably pulmonic stenosis and tricuspid insufficiency. This group was the first to suggest that the cause of clinical and pathologic phenomena might be the secretion by tumor cells of a humoral substance, possibly 5-hydroxytryptamine or serotonin. This suggestion was made on the basis of earlier work, notably that by Lembeck[9] who, in the previous year, succeeded in extracting serotonin from a benign carcinoid tumor and Erspamer[10] who expressed the belief that serotonin was produced by the chromaffin cells of the intestinal tract.

Subsequent reports by Pernow and Waldenstrom,[11] Sjoerdsma and co-workers[12] and Page et al.[13] substantiated this hypothesis, for large amounts of a degradation product of serotonin, 5-hydroxyindol acetic acid, were found in the blood and urine of patients with the syndrome.

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Sjoerdsma, Udenfriend and co-workers[14] have described chemical and colorimetric diagnostic tests based on these findings in blood and urine, and Hanson and Serin[16] have recently described an extremely simple qualitative test.

On first consideration it appears somewhat unexpected to find lesions only of the right side of the heart occurring with the carcinoid syndrome. Gobel and co-workers[17] offer at least a partial explanation for this occurrence and their results are in keeping with observations made at least thirty years previously. They collected blood simultaneously from both the pulmonary and brachial artery in a patient with the syndrome and found that two-thirds of the serotonin in the blood, not bound to platelets, was removed in a passage through the lungs. They pointed out that the high content of mono-amine oxidase in the lung converts the $—CH_2NH_2$ group of 5-hydroxytryptamine to —COOH, producing 5-hydroxyindol acetic acid, a pharmacologically inactive substance. This may supply a portion of the answer; mono-amine oxidase is found in other organs as well and there is a possibility that the liver has a role in serotonin metabolism.

Serotonin. The history of serotonin antedated and later paralleled that of carcinoid tumors without the two becoming associated until the successful work of Lembeck in 1953. In 1884, four years before Lubarsch[18] first described a carcinoid tumor, Stevens and Lee[19] at Johns Hopkins had recognized a vasoconstrictor substance in clotted blood. Earlier observations that serum from clotted blood when perfused through the muscle of dogs encountered or caused an increased resistance to its passage led to this discovery. The subsequent chief interest of pharmacologists and physiologists in the serum vasoconstrictor was its possible role in hypertension and blood dyscrasias, for it was normally present in large amounts in the platelets. In 1925 Starling and Verney[20] observed that perfusion of animal lungs removed the serum vasoconstrictor; in 1950 Bradley et al.[21] demonstrated an enzyme in the pulmonary vascular bed, mono-amine oxidase, as the active agent in this removal. In 1948 the vasoconstrictor substance, 5-hydroxytryptamine, was isolated and crystallized by Rapport, Green and Page[22] and called serotonin. Erspamer, working independently, gave it the name enteramine. Hamlin and Fisher[23] were among the first to report its synthesis. The review articles of Page[24] and Erspamer[25] deal with its effects in experimental animals; there are marked species differences but clinical manifestations of the carcinoid syndrome, namely flushing, bronchoconstriction and gastrointestinal hypermotility, have been reproduced in animals by injections of serotonin.

Previously Reported Cases of the Carcinoid Syndrome. Up to the present time, fifty-three acceptable cases of the carcinoid syndrome have been reported.[8,12,17,26–45] Of nineteen additional cases reported, sixteen appear to be duplicates;[11,13,29] one case was reported in which a biopsy specimen had not been taken to prove the presence of carcinoid tumor;[42] in one case adenocarcinoma of the bile ducts was present, with the finding of Aschoff's bodies in the heart;[41] in another which, with the latter, was included in the collection by Thorson et al. carcinoma of the stomach was noted.

Definite isolated pulmonic and/or tricuspid valve lesions, excluding rheumatic valvular lesions, were present in thirty-one of these fifty-three cases.[8,16,26,28,32,34,35,37,38,39,41,43] An additional subject with involvement of all four valves had patent foramen ovale.[44] Not all the cases reported have been subjected to autopsy as yet.

This study was undertaken with two purposes: first, to study certain aspects of the natural history of gastrointestinal carcinoids and, second, to determine whether or not cases with clinical and pathologic features of the carcinoid syndrome could be found in Boston hospitals.

MATERIAL AND METHODS

Up to the present time, the largest number of patients with carcinoid reported by one author has been 140.[46] The present series of 356 comprises 202 from the Mallory Institute of Pathology and 154 from six other Boston hospitals. The 202 from this institute comprise seventy-two occurring in 18,486 consecutive autopsies (1934–1955 inclusive) and 130 occurring in 114,787 consecutive surgical specimens (1910–1955 inclusive). At outside hospitals 154 carcinoids are reviewed, made up of 124 occurring in 303,379 consecutive surgical specimens, and thirty occurring in 7,915 consecutive autopsies (1934–1955 inclusive).

The pathology records of all cases were read and histologic sections from 101 extra-appendiceal carcinoids, those available, were reviewed. In cases at the Boston City Hospital, stored tissue from patients with metastases to distant organs was reviewed as well. A gradation of tumor invasiveness was made, using gross and microscopic findings, as follows: (1) non-invasive,

TABLE 1

ONE HUNDRED FORTY-NINE EXTRA-APPENDICEAL CARCINOID TUMORS OF THE GASTROINTESTINAL TRACT OCCURRING IN 26,401 AUTOPSIES AND 418,166 SURGICAL SPECIMENS AT SEVEN BOSTON HOSPITALS

Site	Non-invasive	Invasive			Could Not Be Determined	Totals
		Muscle Layer	Nodes	Distant Organs		
Stomach	4	2	6	2	..	14
Duodenum	5	2	0	1	..	8
Jejunum	7	4	4	2	..	17
Ileum	26	24	15	8	1	74
Cecum	2	0	1	1	1	5
Colon	0	2	2	1	1	6
Sigmoid	0	0	1	1	..	2
Rectum	2	5	3	3	..	13
Gallbladder	0	1	0	0	..	1
Meckel's diverticulum	2	0	0	0	..	2
? primary	0	0	2	5	..	7
Totals	48	40	34	24	3	149
% of 146*	33	27.3	23.3	16.4	..	100

* Three cases could not be graded.

those carcinoids confined to submucosa and mucosa, with no invasion of underlying muscle coats of bowel wall or of lymphatics; (2) grade 1, those with invasion of underlying muscle coats or of lymphatics; (3) grade 2, those which had spread to lymph nodes or had broken through the serosa of the bowel to abut on adjacent structures; (4) grade 3, those which had invaded blood vessels or had spread to organs other than lymph nodes, such as liver.

Histologic sections of patients with appendiceal carcinoids were reviewed only in those cases in which the tumor had been reported to show some degree of invasiveness when diagnosed microscopically.

The clinical records of patients at the Boston City Hospital whose tumors had spread to lymph nodes or other organs were searched for clinical features of the carcinoid syndrome as well as for other findings in common. At outside hospitals, only the clinical records of patients with metastases to distant organs were reviewed.

As relative control groups, to establish the frequency of pulmonic and tricuspid valve lesions in conditions other than carcinoid tumors, the following were studied: (1) All cases of congenital pulmonic stenosis occurring in autopsies (1934–1955) at the Boston City Hospital were reviewed, using records and slide material. (2) In the same series, 628 cases of rheumatic heart disease were reviewed at the Boston City Hospital. Autopsy records were read and abstracted and valvular deformities were noted. (3) A similar review was made of 1,000 random autopsies, chosen from these years, excluding cases with rheumatic heart disease and excluding autopsies on persons less than eighteen years of age.

RESULTS AND OBSERVATIONS

Of the total 356 tumors in this series, 149 were extra-appendiceal. The relevant data concerning these are summarized in Table 1.

In past writings, carcinoids from appendiceal and extra-appendiceal locations have often been grouped together in citing incidence of malignancy. It seems justifiable to treat the two groups separately inasmuch as the natural behavior in each location is strikingly different.

Up to the present time there have been no definite criteria for malignancy in carcinoid tumors on the basis of cytology. It is clear from most writings on the subject, however, that spread to lymph nodes and other organs has been the criterion most often applied. The findings in this series are in agreement with prior reports[48–50] that the usual standards of tumor malignancy, such as mitoses and cell anaplasia, are not applicable to carcinoids. The majority of patients in whom metastasis to lymph nodes and other organs had occurred rarely had evidence of mitosis or anaplasia. Because of this, malignancy in carcinoids must be determined on the basis of gross and microscopic invasiveness. A further basis for such a system is the following observation. In all but one of the cases in this series, in which carcinoid tumor had spread to lymph nodes or other organs and histologic sections or, in their absence, adequate microscopic descriptions were examined, the primary

tumor showed invasion of underlying muscle coats and lymphatics. In most cases it had spread through the bowel wall to reach and break through the serosal coat. Further, in all cases in which the liver and other distant organs were involved, regional lymph nodes adjacent to the primary carcinoid tumor had been involved. It therefore seems probable that malignant carcinoids follow a progressive pattern of invasion, first invading underlying muscle coats, reaching lymph nodes by lymphatic spread and passing to liver by blood vessel invasion. This appears to take place in a step-wise manner.

Using these criteria, invasiveness in 146 extra-appendiceal carcinoids that could be graded was as follows. Thirty-three per cent were non-invasive, 27.3 per cent invaded muscle coats only, 23.3 per cent had spread to regional lymph nodes, and 16.4 per cent had spread to distant organs. In all, 67 per cent showed invasiveness and 39.7 per cent had spread to lymph nodes and beyond.

The fact that carcinoids tend to be multiple in origin has been noted in many prior writings. Of 149 extra-appendiceal carcinoids in this series, 16 per cent were of proved multiple origin.

Appendiceal Carcinoids. Of the 356 carcinoids in this series, 207 (58 per cent) were appendiceal in location. This contrasts with the figure of 90 per cent for appendiceal location usually cited in the literature.[50] The predominance of appendiceal carcinoids in surgical as opposed to autopsy material has been noted in the past[46] and proved to be the case at all hospitals consulted.

One unresolved feature of appendiceal carcinoids concerns their malignancy. There is agreement that they spread to lymph nodes and other organs with far less frequency than extra-appendiceal carcinoids, but how often and how far they spread has never been reliably determined. The same criterion for malignancy, namely invasiveness, is not as applicable to carcinoids in the appendix as to those elsewhere in the gastrointestinal tract.

On reviewing the literature, one is struck by the fact that many appendiceal carcinoids reported as malignant are so poorly documented as to make the designation of malignancy unacceptable. When a carcinoid tumor is found in the appendix and in lymph nodes or other organs, the assumption is often made that the appendix was the primary site for the metastasis without search for another extra-appendiceal site and without microscopic demonstration of local invasion by the appendiceal tumor.

The following criteria have been used to establish malignancy in appendiceal carcinoids: (1) microscopic demonstration of tumor invasion into underlying muscle coats, lymphatics or blood vessels and (2) exclusion of possible extra-appendiceal primary sites for a metastasis. Using these criteria, only thirteen acceptable cases of "malignant" appendiceal carcinoids have been found in the English literature, using malignant to mean invasive. Of these, two[51,52] involved only underlying muscle coats, three[53–55] had broken through the serosal coat of the appendix, six[53–58] had grown to involve mesoappendix or peritoneum, and two[58,59] had spread to involve regional lymph nodes. No acceptable case was found, according to these criteria, with spread beyond regional lymph nodes or adjacent structures.

Of the 207 appendiceal tumors in this series, twenty-three (11 per cent) showed invasiveness and met the standards outlined. Eleven invaded muscle coats, ten had broken through the serosal coat of the appendix, two had grown to mesoappendix or peritoneum, and none had metastasized to regional lymph nodes. In no case was there spread to distant organs. From this review it appears that appendiceal carcinoids are not implicated in the carcinoid syndrome inasmuch as they do not produce significant metastases.

Control Groups and Cases of the Carcinoid Syndrome. In 18,486 consecutive autopsies, isolated pulmonic stenosis occurred five times, an incidence of 0.03 per cent.

In this same number of autopsies, involvement of the right side of the heart alone was evident in only one of 628 cases of rheumatic heart disease, an incidence of 0.16 per cent. In this one case the tricuspid valve cusp was deformed, with shortening and fusion of the chordae tendineae. In no instance was the pulmonic valve alone affected or the tricuspid and pulmonic valves involved together. Clawson[60] in 779 cases of autopsied rheumatic heart disease found pulmonic valve involvement twice, an incidence of 0.25 per cent, and no cases of tricuspid valve involvement. With this exception and with the difference that among cases at this institute the aortic valve alone was affected in only 4 per cent of cases (compared with 41 per cent of cases in Clawson's series) our results are in agreement.

In the 1,000 random autopsies taken from the

same autopsy population, 1934–1955, excluding rheumatic heart disease and excluding autopsies of persons under eighteen years of age, 50.2 per cent showed valvular or endocardial deformities. There was no occurrence of pulmonic stenosis. An isolated pulmonic valve lesion was found only once; this was a four-cusped valve without stenosis. An isolated tricuspid valve lesion occurred twice, and pulmonic and tricuspid lesions combined occurred once. These changes consisted of edematous thickening of the valve cusps, thickening of the chordae tendineae and calcification in the tricuspid valve ring. The total occurrence of lesions on the right side of the heart, without valvular lesions of the left side of the heart, was four times (0.4 per cent). The autopsy population of this institute is drawn predominantly from middle aged and elderly persons, but a large number of youthful persons in this series of 1,000 random autopsies showed findings on the left side of the heart. These were usually described as fibrous thickenings or fibrous nodularities of valves and as hyaline nodularities of valve cusps or endocardium.

Of 149 extra-appendiceal carcinoid cases in this series, widespread metastases were present in twenty-four; all but one involved the liver. The records of twenty-one of these twenty-four cases were available and suitable for review. Three definite cases and one probable case of the syndrome were found in this group, all with liver metastases. Another case had certain clinical features but at autopsy showed no cardiac findings.

Two of the definite cases occurred in autopsies at the Boston City Hospital, culled from eight cases of widespread metastases. Clinical features of the syndrome were present in both, namely, skin changes, diarrhea, a large liver and cardiac murmurs. At autopsy, pulmonic stenosis was observed. An interesting case included in these eight autopsies was one of acropachyderma and pachyperiostitis which was reported in the medical literature seven years before death.[61] At postmortem examination, there were no relevant cardiac findings; there was marked thickening of the skin and bone of fingers and toes as well as thickening of the skin of the face.

The third definite case, currently under study, is reported through the courtesy of Dr. Harold Rheinlander, of the New England Medical Center. The patient has metastatic carcinoid tumor of the liver as demonstrated at laparotomy. He has frequent episodes of marked flushing,

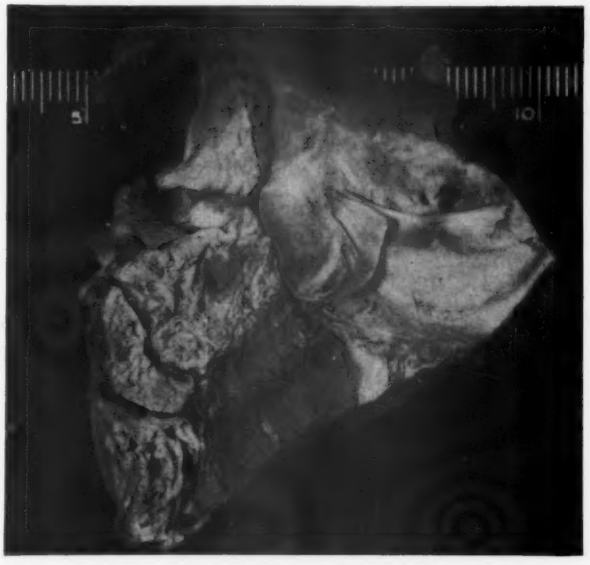

FIG. 1. Case I. Relatively normal aortic valve cusps.

occurring especially at defecation, when urinating and after meals. No cardiac murmurs have been noted and other features of the syndrome are lacking. A qualitative test for 5-hydroxyindol acetic acid in the urine is positive.

The probable case, a patient who was diagnosed clinically at two separate hospitals, showed reddening of the skin, diarrhea, a large liver, metastatic carcinoid tumor to the liver, and isolated tricuspid valve involvement. There was no past history of rheumatic fever.

The case which possibly belongs in the group exhibiting the syndrome was autopsied at another hospital. This patient had had a constant flush of the skin, diarrhea, a murmur at the pulmonic area and a large liver. At postmortem there was metastatic carcinoid to the liver but no relevant cardiac findings.

CASE REPORTS

CASE I. A. C., a forty-six year old white housewife, entered Boston City Hospital (No. 1227188) for the first time four days before death. For a month and a half before admission she had noted swelling of her legs and abdomen, dysentery consisting of ten to twelve watery, painless bowel movements daily and frequent episodes of chilly sensations alternating with drenching sweats. About six months before entry she had noted painless stiffening and swelling of her fingers. Nine months earlier she had sought medical attention for menopausal symptoms consisting of episodes of flushing of the face accompanied with a sensation of warmth. During the past year she had lost approximately twenty-five pounds. For the previous five years she had had a rash over her cheeks, nose and

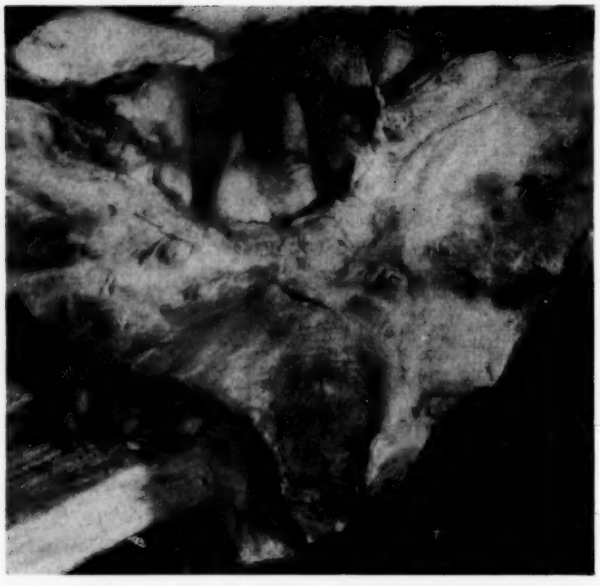

FIG. 2. Case 1. Ventricular aspect of pulmonic valve. Note short, thick, rigid valve cusps with interadherence at commissures.

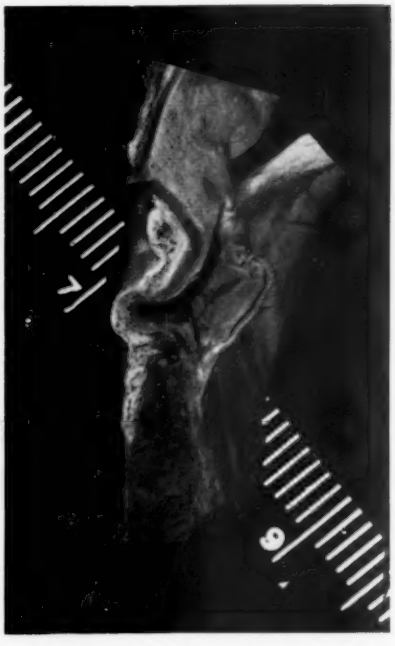

FIG. 3. Same case. Cut surface view of pulmonic valve cusp and pulmonic artery.

chin. She admitted to moderate alcohol intake for the past fifteen to twenty years but had stopped drinking altogether two years earlier. She did not give a reason for stopping.

Her family history was not contributory; her father had had heart disease.

Physical examination revealed a poorly nourished, chronically ill appearing white woman, able to give a history but with memory defects of questioned reliability. Vital signs were as follows: pulse 80, respirations 30, temperature 96°F., blood pressure 112/80, weight 120 pounds. The skin of her cheeks, bridge of nose and chin was described by one observer as "thickened and erythematous with telangiectasia" and as "a reddish violet-blue rash with marked telangiectasis" by another. The hands and fingers showed "fusiform swelling of the interphalangeal joints, with some limitation of motion" and "reddening." The lungs were clear. There was a harsh grade 2 systolic murmur over the precordium and the heart was slightly enlarged. The neck veins were distended. The abdomen was distended, contained fluid and was diffusely tender. The liver could not be felt. There was 2+ pitting edema of the lower extremities.

Laboratory studies included the following: red blood count 4.2 million, hemoglobin 72 per cent of normal; white blood count 9,000 cm. with 80 per cent polymorphonuclear leukocytes, 18 per cent lymphocytes, 2 per cent eosinophils; the urine was acid with 2+ albumin and occasional white blood cells and granular casts per high power field. The serum nonprotein nitrogen was 92 mg. per cent, total protein 5.6 gm. per cent; Hinton reaction negative; stool guaiac reaction negative; cephalin flocculation test was 2+, direct bilirubin 1.19 mg. per cent, total bilirubin 2.08 mg. per cent; the peripheral blood and sternal marrow contained nucleated red blood cells; the venous pressure was 12 cm. of water, the circulation time thirty-five seconds.

The patient was given blood transfusions and digitalized but remained weak and stuporous. On the third day jaundice developed (icterus index 50); on the fourth day a paracentesis was performed, following which the liver could be felt approximately 6 cm. below the central costal margin. Several hours after this procedure she became progressively weaker, lapsed into coma and died.

At autopsy (No. A47-104) malignant argentaffinoma, primary site in the terminal ileum, was found with metastases to mesentery, regional nodes, pancreas and nodes, heart, spleen, ovaries, adrenals, bone marrow and massively to the liver. There was cirrhosis of the alcoholic type and stenosis of the pulmonary valve. The liver weighed 2,900 gm.; the heart weighed 300 gm. A metastatic nodule was located beneath the epicardium of the left ventricle, one in the septum and a third in the wall of the right ventricle. The endocardium was smooth, the valves all delicate except for the pulmonic valve which was remarkably small, measuring 4.5 cm. in circumference. The cusps were small and interadherent. The sinuses of the first portion of the pulmonary artery were unusually deep. The first portion of the pulmonary artery was narrowed but immediately widened to a normal diameter. The wall of the right ventricle was slightly thickened, measuring 0.4 cm.

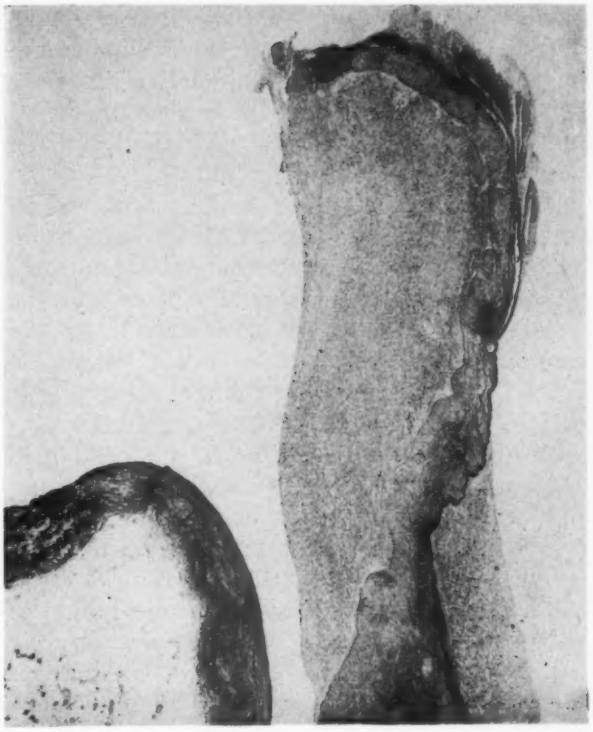

FIG. 4. Same case. Pulmonic valve cusp and portion of pulmonic artery. Note relatively normal valve cusp on right with overlaid layers of fibrous tissue present on both ventricular and artery surfaces. The overlaid fibrous tissue does not take the stain. Elastic tissue of pulmonary artery is normal. Verhoeff's elastica stain. Original magnification, × 30.

FIG. 5. Higher magnification of pulmonic valve cusp. Ventricular edge of cusp is on right. Endothelium of cusp is intact and fibrous tissue is deposited upon it. Verhoeff's elastica stain. Original magnification, × 100.

The aortic valve measured 7.0 cm. in circumference, the tricuspid 7.0 cm., the mitral 8.0 cm. and the left ventricle measured 0.8 cm. in thickness.

CASE II. H. H., an eighty year old white widow, was admitted to Boston City Hospital (No. 1275408) for the first time, from a nursing home, because of stupor of two days' duration. She had been in good health until five months before admission when she began to have severe diarrhea progressing to incontinence of feces and was placed in the nursing home. She had also lost weight since that time, amount unknown.

Scanty information was available about her past history other than a daughter's statement that the patient had enjoyed good health prior to symptoms.

Physical examination revealed a florid-faced, cachectic, elderly white woman in semi-coma, responding feebly to pain. Vital signs were as follows: pulse 118, respirations 22, temperature 101°F., blood pressure 84/40. The skin was described by two observers as having a "striking redness," and "a reddish cyanotic hue." The head was kept turned to the right, as were the eyes; there was a lower right facial droop and both plantar responses were equivocally abnormal. The heart was enlarged, with apical and basal systolic murmurs. The chest was clear except for occasional basal rales. The liver was felt 5 cm. below the costal margin and was hard and nodular. There was 3+ pretibial and sacral edema.

Laboratory tests included the following: hematocrit 36 per cent, white blood count 7,600 with 82 per cent polymorphonuclear leukocytes, 16 per cent lymphocytes and 2 per cent monocytes; the urine was acid with 1+ albumin and 3 to 5 hyaline and granular casts per high power field; the Hinton reaction was negative, serum non-protein nitrogen 74 mg. per cent, icteric index 12.5 units, cephalin flocculation test 1+, direct bilirubin 0.21 mg. per cent and total bilirubin 1.20 mg. per cent; a stool guaiac reaction was 2+ positive; a lumbar puncture yielded clear fluid with a total protein of 36 mg. per cent. Despite penicillin, oxygen and parenteral feedings, she lapsed into coma and died on the third hospital day.

At autopsy (No. A48-153) there were multiple carcinoid tumors of the jejunum, a single tumor nodule in the stomach and massive metastases to the liver. There was pulmonic stenosis with tricuspid valve thickening. The liver weighed 1,980 gm. and showed marked fatty change. The heart weighed 350 gm.; atherosclerotic plaques were noted in the aortic valve leaflets. The mitral valve was normal, measuring 7.0 cm. in circumference. The pulmonic

valve was stenosed, with a circumference of 5.0 cm. Its leaflets were thickened, rigid and interadherent. The aortic valve circumference was 5.9 cm. The base of the tricuspid valve was slightly thickened, with slightly thickened chordae tendineae. Its circumference was 8.5 cm. The left and right ventricular thicknesses were 1.3 cm. and 0.4 cm. Neuropathologic findings to explain this clinical picture were not found, other than severe atherosclerosis of cerebral arteries.

TABLE II
MANIFESTATIONS OF FEATURES OF THE CARCINOID SYNDROME IN TWENTY-ONE CASES OF CARCINOID TUMORS OF THE GASTROINTESTINAL TRACT WITH WIDESPREAD METASTASES

	Skin	Joints	Large Liver	Broncho-constriction	Diarrhea	Ulcers
8 autopsies*.....	3	4	7	0	5	3
Total 21 cases...	4	6	19	2	9	8

* Performed at the Mallory Institute of Pathology and included in the breakdown of the twenty-one cases.

The concurrence of congenital anomalies with the carcinoid syndrome has been remarked upon.[43] In the twenty-one cases of widely metastatic tumor, anomalies occurred three times, cleft palate once and Meckel's diverticulum twice. In addition, a case with acropachyderma with pachyperiostitis was found. None of these were in patients with this syndrome.

Joint involvement occurred in four of the eight autopsies at the Mallory Institute of Pathology and in six of the twenty-one cases reviewed. In all cases there was arthritic change of the finger joints with additional knee involvement in one of the cases. All six patients were in the age range in which degenerative joint changes are common.

Peptic and duodenal ulcers were noted in three of the eight autopsies at the Mallory Institute of Pathology and in eight of the total twenty-one cases that could be reviewed. In four cases the diagnosis was made by autopsy or surgical specimen, in three by gastrointestinal series and in one by history and response to therapy. (Table II.)

On reviewing the clinical records of the twenty-one patients with metastases it appeared that an unusual degree of mental disturbance had been noted by the examiners. The patients were described as uncooperative, unreliable, contradictory and very nervous, with a large functional component to their illness. These observations do not have the same significance in seriously ill persons as in relatively healthy persons, but suggestions have been made[62,63] that serotonin may have a physiologic role in central nervous system function.

Pathology. In addition to the tumor and its metastases, the pathologic findings in the two autopsies of persons with the carcinoid syndrome were of note in two areas, the liver and the heart. In the liver, areas removed from the tumor as well as those adjacent showed a marked degree of fatty change. Liver tissue reviewed from twenty of the remaining cases with wide metastases was relatively normal in all but one. Only the case of acropachyderma and pachyperiostitis showed a comparable but still significantly less degree of fatty infiltration.

The findings in the heart in both cases were of pulmonary stenosis but not of the congenital or rheumatic type. Case 1 was the most severely involved. (Figs. 1 to 3.) Grossly, the valve cusps were shortened, thickened and rigid, with interadherent commissures. Visible beneath the endocardium on the ventricular surface, below and on the pulmonic valve cusps, were fibrous linear thickenings, running obliquely upward toward the valve. Microscopically, special stains delineated the valvular process. With ordinary hematoxylin and eosin stains the valve cusps appeared diffusely thickened and fibrous, with large vascular channels passing through the valve cusps. With phloxine-methylene blue or Giemsa stains many tissue mast cells were seen in the areas of increased vascularity of the cusps. The most striking features were brought out by use of elastic tissue stains. These showed the valve cusps to be relatively normal with intact elastic tissue and endothelium upon which loose fibrous tissue was overlaid in a manner suggesting concentric layers. This fibrous tissue was present on both ventricular and pulmonary artery aspects of the cusps. (Figs. 4 and 5.) The fibrous tissue was somewhat unusual in appearance, characterized by a predominance of intercellular ground substance and suggesting an active fibrous process; it did not contain elastic tissue fibrils. (Fig. 6.) The pulmonary artery contained the usual amount and extent of elastic tissue, lending support to the belief that this was not a congenital stenosis. The small arteries of the lungs showed a slight but definite degree of hyperplastic thickening of the walls.

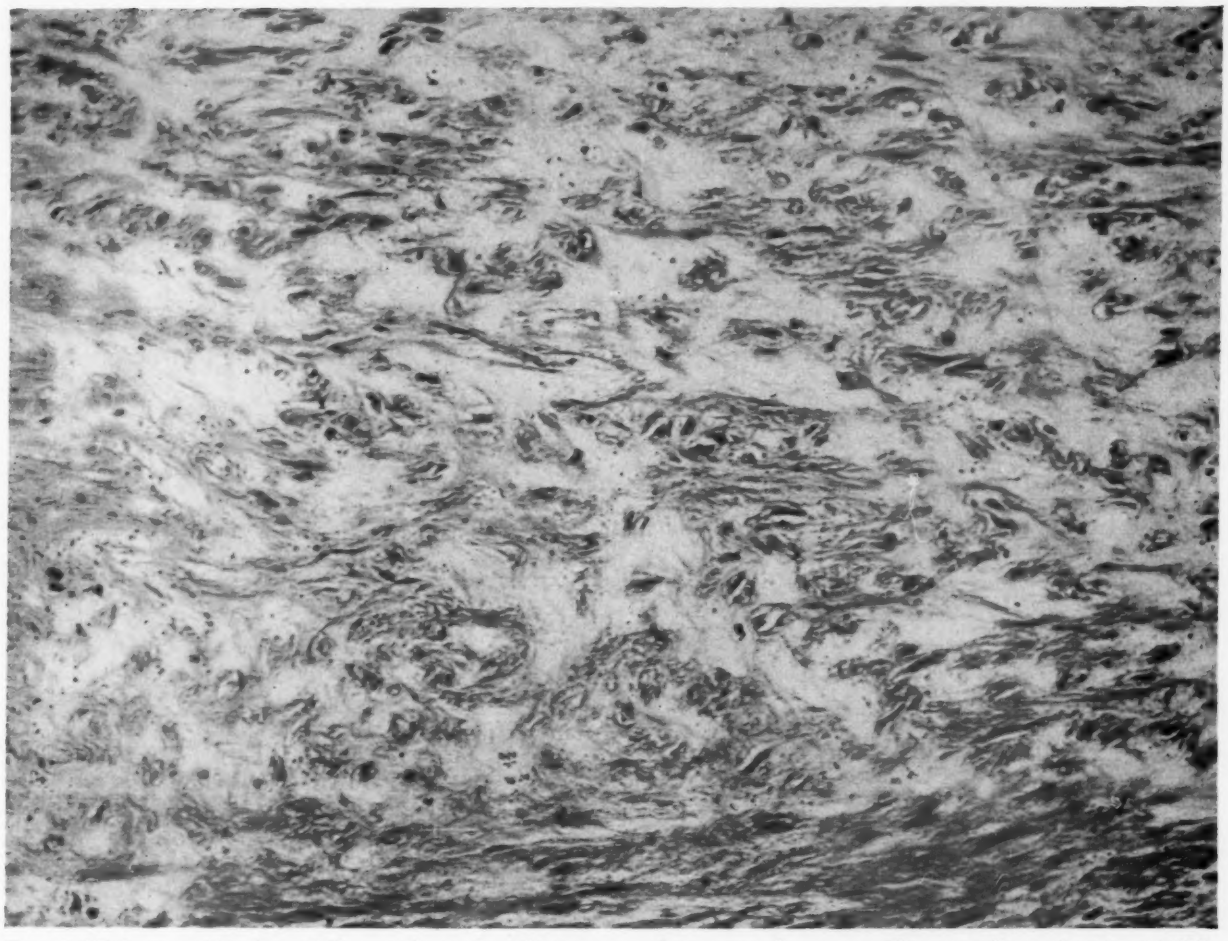

FIG. 6. High magnification of fibrous tissue deposited upon pulmonary valve cusps. Hematoxylin and eosin, × 400.

COMMENTS

From experience with this series of cases and a review of the literature, extra-appendiceal carcinoids are considered to be low-grade malignant tumors which in most cases spread slowly and progressively. A carcinoid invading only muscle coats of bowel is not considered benign but has probably been found in the first stage of progressive spread. It would be preferable for the pathologist to report extra-appendiceal carcinoids not as benign or malignant, but in terms of invasiveness, namely, (1) non-invasive, confined to submucosa and mucosa; (2) invading muscle of bowel wall; (3) invading lymphatics or in regional nodes; (4) invading blood vessels or with spread to distant organs. The surgical implications of each of these gradations are important because of the remarkably slow and progressive spread of these tumors; findings at operation must be combined with the microscopic report. For the non-invasive type and the carcinoid invading muscle coats of bowel only, local resection is probably adequate in the absence of lymph node involvement. For the tumor with spread to nodes, local resection of the primary tumor plus local lymph node dissection is advised. For the carcinoid with spread to distant organs, such as the liver, local resection of the primary tumor plus local lymph node dissection, if feasible, is advised. The reason for the latter procedure is the finding in this series and in the literature of a slow rate of progression of carcinoids with liver metastases and the development of local complications at the primary tumor site, particularly obstruction, if the primary tumor is not removed.

The tendency of carcinoids to be multiple in origin is important to the surgeon, for with the finding of a carcinoid at operation others should be looked for. In this series one patient, dying of metastatic carcinoid from the ileum, had had an incidental appendiceal carcinoid

removed four years earlier. Malignancy in carcinoids has been found in persons as young as sixteen years of age.[64]

Of incidental interest is the fact that this series adds twelve stomach carcinoids to the twenty-seven previously reported,[43,48] one gallbladder carcinoid to the four previously reported and two carcinoids in a Meckel's diverticulum to the nine in the literature. Snow[42a] has recently described carcinoid tumor metastatic to the liver with only a carcinoid in a Meckel's diverticulum found at autopsy. There was, however, no demonstration of muscle invasion at the primary site, nor of regional lymph node involvement to substantiate this as the primary site for the metastasis. A similar surgical case has been reported by Grimes and Crane.[42b]

The designation of appendiceal carcinoids as benign or malignant on the basis of invasiveness does not carry the same implications as for extra-appendiceal carcinoids or for other tumors. No appendiceal carcinoids in this series had spread beyond the meso-appendix, and no acceptable case in the English literature had spread beyond regional lymph nodes. Removal of the appendix should therefore be adequate; local lymph nodes, if involved, may be dissected. If there is spread to distant organs, such as the liver, another primary site for the metastasis should be sought. As mentioned previously, appendiceal carcinoids are not considered to be implicated in the carcinoid syndrome.

SUMMARY

1. A series of 356 gastrointestinal carcinoids is presented. Two hundred seven (58 per cent) were of the appendix and 149 (42 per cent) were extra-appendiceal in location.

2. Of 146 extra-appendiceal carcinoids in this series that could be graded, invasiveness was found in 67 per cent. Of the total 149 extra-appendiceal carcinoids, twenty-four (16 per cent) had metastasized to the liver or lungs.

3. The suggestion is made that all extra-appendiceal carcinoids be considered malignant and reported in terms of invasiveness. The surgical implications of these tumors are different than for other gastrointestinal carcinomas and are discussed. Appendiceal carcinoids are of an unusually low grade of invasiveness; no adequately documented case was found in a review of the English literature and in this series of a metastasis beyond regional lymph nodes.

4. Evidence is presented that a definite relationship exists between widely metastatic carcinoid tumors, certain clinical phenomena and valvular lesions of the right side of the heart. The term "carcinoid syndrome" is suggested as a simple term to denote this group of findings.

5. Evidence is presented that non-specific gross lesions of the left side of the heart cannot be implicated in the carcinoid syndrome.

6. Of twenty-four cases in this series with liver or lung metastases, twenty-one could be clinically as well as anatomically reviewed, and four (19 per cent) exhibited the carcinoid syndrome. The addition of these new cases brings the total number reported to date to fifty-seven. Two of these cases had proved isolated pulmonic stenosis. These bring the total number of cases with proved isolated non-rheumatic lesions of the valve of the right heart, (or combined lesions on the right and left side in the event of patent foramen ovale) to thirty-four.

7. Gastric and duodenal ulcers, not previously mentioned in connection with metastatic carcinoid, were found in eight of the twenty-one cases (38 per cent). By comparison, the incidence of all types of gastric and duodenal ulcers in 18,486 consecutive autopsies was 5.5 per cent. The significance of an additional finding, of arthritic changes usually of the joints of the fingers, must still be investigated.

8. It is concluded that not all patients with extensive carcinoid metastases exhibit the features of the carcinoid syndrome and not all patients with the syndrome exhibit all the features previously described. The most constantly occurring appear to be the tumor and its liver metastases, episodes of flushing or a constant flush of the skin, a large liver and diarrhea.

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A Consideration of Chronic Pulmonary Parenchymal Inflammation and Alveolar Cell Carcinoma with Regard to a Possible Etiologic Relationship*

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THE identification of lesions which are precancerous or which predispose to neoplasia is of obvious importance. In this presentation the concept is advanced that chronic pulmonary parenchymal inflammation represents such a lesion by laying the groundwork for the later development of so-called alveolar cell tumors.

Alveolar cell tumors are primary pulmonary neoplasms arising in the peripheral portions of the lung and characterized by cuboidal or tall columnar epithelial cells lining alveolar septums with no associated desmoplasia. Mucus production frequently occurs but is not constantly present. Absence of an intrinsic bronchial tumor or of a primary adenocarcinoma elsewhere in the body is prerequisite for absolute diagnosis, although the classic histologic appearance of alveolar cell carcinoma is not duplicated by any other tumor which occurs in the lung.

It has been customary to differentiate between pulmonary adenomatosis and alveolar cell carcinoma but the similar histologic picture renders such a separation meaningless. The only distinction is that one tumor extends locally while the other possesses the additional capability of metastasis by lymphatics and blood stream. A more logical approach would be to consider pulmonary adenomatosis and alveolar cell carcinoma as representing the same neoplasm with variable malignancy. Analogous situations are seen in other tumors, for example, certain cystadenomas of the ovary may spread only locally by implantation over the peritoneum while others, similar microscopically, metastasize through the lymphatics and blood stream in addition to exhibiting the peculiar local seeding effect.

In this article these tumors will be designated as alveolar cell carcinomas because of established usage, although the term "bronchiolar carcinoma" is now acquiring favor. The site of origin of these tumors—whether from the much debated alveolar epithelium or from the bronchiolar epithelium—is not under consideration.

The present study is concerned with seven cases of typical alveolar cell carcinoma and one case exhibiting the microscopic changes associated with this condition, apparently on the basis of metaplasia of epithelium subsequent to chronic inflammatory disease. The latter case is of particular interest in that early changes indicative of the lesion are present and it offers convincing evidence concerning the relationship of chronic pneumonitis and alveolar cell carcinoma. These and all previously reported cases suitable for analysis have been critically reviewed with reference to the existence of antecedent infection in the lung as a predisposing factor in the development of the neoplasm. This analysis suggests a possible explanation for the apparent increase in the incidence of alveolar cell carcinoma.

In classifying the cases under discussion as belonging to this peculiar group of pulmonary neoplasms, the usual diagnostic criteria have been utilized as far as practicable. In four of the patients the presence of a primary neoplasm elsewhere in the body was excluded by autopsy and in the remaining living patients there has been no clinical evidence of an extrapulmonary neoplasm. No endobronchial tumor was found in the lungs removed surgically or at autopsy in any of the eight cases. The first two cases represent the more malignant variety of the tumor, extra-

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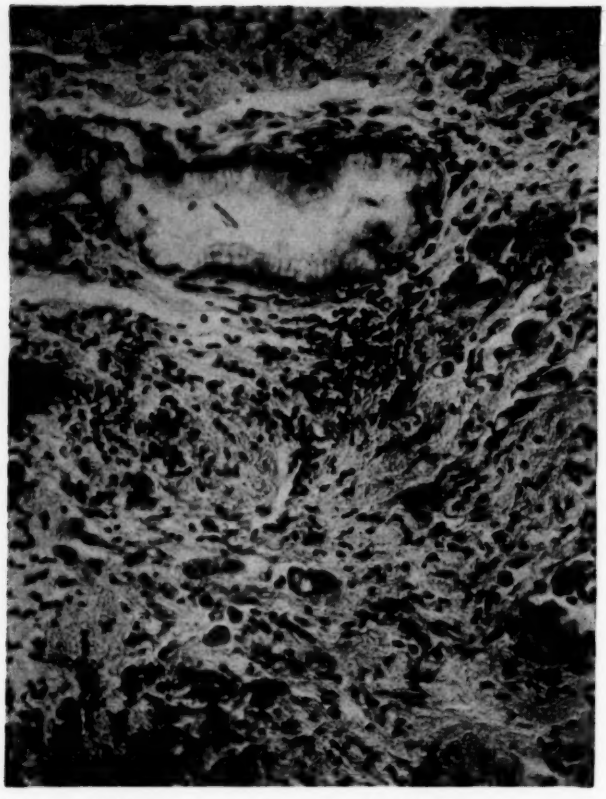

FIG. 1. Adenomatous area, chronic inflammation and invasion of stroma by anaplastic tumor cells.

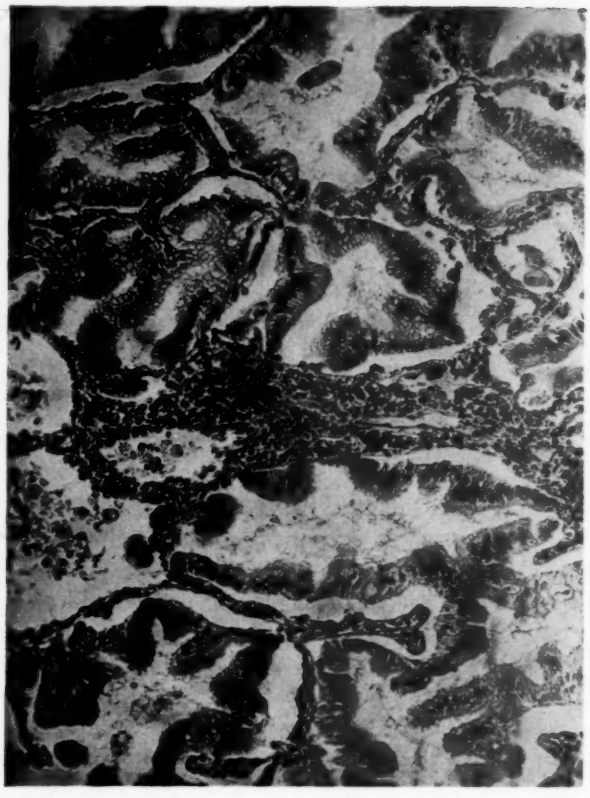

FIG. 2. Case I. Alveoli lined by tall columnar cells in the typical pattern of alveolar cell carcinoma.

pulmonary metastases being present. In the remaining cases the tumor was confined to the lungs.

CASE REPORTS

CASE I. (V. U. H. No. 74728.) A fifty-two year old white man was first admitted to Vanderbilt University Hospital in 1935. Three weeks before admission he had had a sudden onset of febrile illness with severe sweats, cough, sputum and bilateral pleuritic pain. An abscess in the right lower lobe of the lung was treated by postural drainage. During the intervening years the patient had intermittent bouts of fever approximately every six months and was treated with arsenicals, sulfanilamide and sulfapyridine. About one and one-half years prior to his last admission in 1941 there was a definite change in his clinical course. Cough and sputum increased and the patient complained of malaise, weakness and drowsiness. He died nine days after admission to the hospital.

Autopsy revealed a collapsed right lung with marked bronchiectasis, considerable fibrosis and an adhesive pleuritis. Small, white, confluent and scattered tumor nodules were present bilaterally with massive involvement of the right lower lobe. There was no tumor in the dilated bronchi which were filled with pus.

Microscopic examination of sections from the right lower lobe showed marked fibrosis and chronic inflammation with anaplastic tumor cells in the chronically inflamed tissue bordering a bronchiectatic cavity. (Fig. 1.) Many cystic spaces were lined by columnar epithelial cells with pink-staining cytoplasm and dark, basally placed nuclei. Mitotic figures were occasionally seen. In other areas the tumor cells were confined to the alveoli. (Fig. 2.) A single metastasis was found in a regional lymph node. A small area of lipoid pneumonia was present in the left lung.

CASE II. (V. U. H. No. 235,705.) A sixty-two year old white man was apparently in good health until six weeks before admission to Vanderbilt University Hospital in July, 1954. The chief complaint was pain in the region of the right scapula. The patient had been examined six months before and was said to be in good health. The past history was significant in that twenty-five years previously he had had drainage of an empyema in the right posterior thorax. He had smoked one pack of cigarettes per day for many years. A roentgenogram showed a rounded homogeneous opacity at the level of the fourth and fifth ribs posteriorly in the right lung. Bronchoscopic examination revealed no tumor and bronchial washings were reported as free of tumor cells. An exploratory thoracotomy was performed. The right upper lobe and a portion of the chest wall to which the tumor was fixed were removed. A pneumonectomy was not

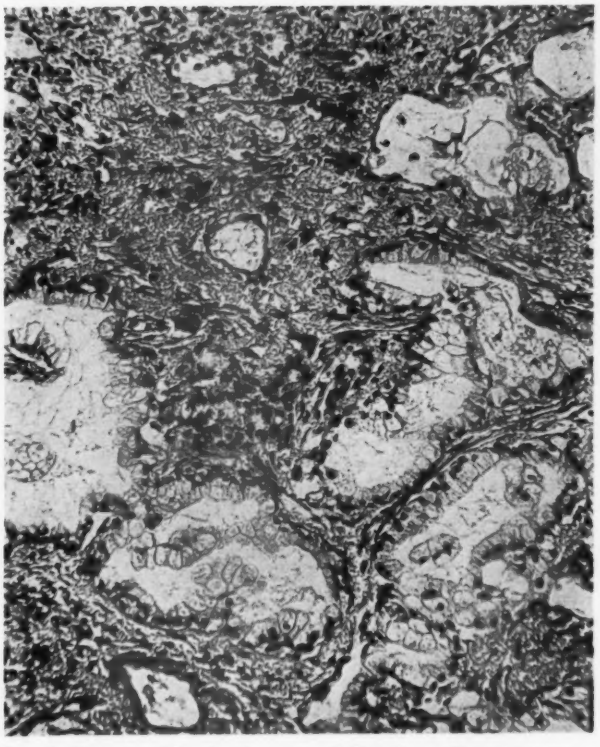

FIG. 3. Case III. Classic changes of alveolar cell carcinoma in an area of organizing pneumonia.

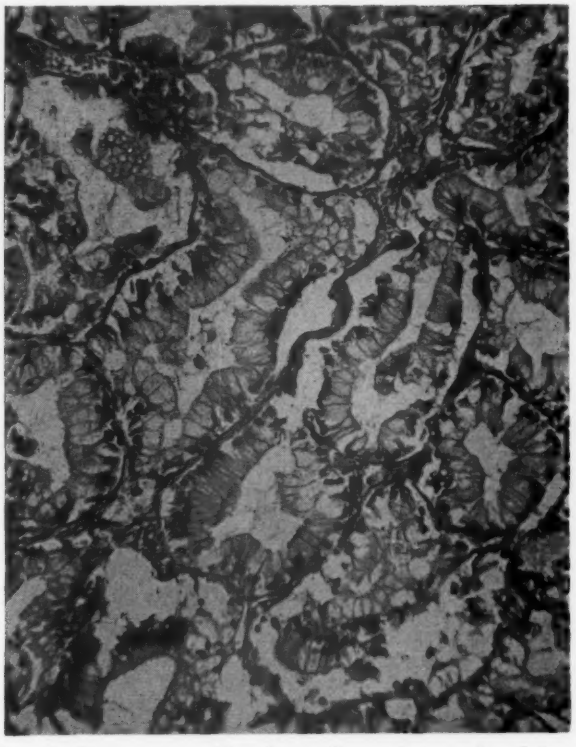

FIG. 4. Same case. Alveolar cell carcinoma involving another area without chronic inflammatory reaction.

performed because of the presence of bilateral emphysema.

Grossly the tumor was greyish white. One of the excised ribs was invaded by the tumor, presumably by extension through pre-existing pleural adhesions. No evidence of endobronchial disease was noted. Microscopically, the tumor consisted of eosinophilic non-ciliated columnar epithelial cells which were thrown into papillary projections and lined the dilated terminal bronchioles and alveoli. Marked septal thickening secondary to fibrosis and a massive infiltration of chronic inflammatory cells were observed. No lymph node metastases were demonstrated.

Postoperatively empyema developed and the patient died in spite of drainage and intensive antibiotic therapy. The autopsy revealed an acute bacterial endocarditis with infarctions of the spleen and kidneys. No residual tumor was found.

CASE III. (V. U. H. No. 202,139.) This seventy year old white married woman died at home four months after the last of three admissions to the Vanderbilt University Hospital. The patient was first admitted in August, 1951, for pneumococcal pneumonia of the right lower lobe and was treated with penicillin and chlortetracycline. Although the patient improved, evidence of unresolved pneumonitis and atelectasis was observed eleven days later at the time of discharge. She was again admitted in February, 1954, with weakness, myalgia, a 25 pound weight loss and a chronic cough productive of frothy sputum. Roentgenograms showed atelectasis of the right lower lobe as well as fan-shaped densities bilaterally. Sputum cultures again were positive for pneumococci and she received massive doses of penicillin with symptomatic improvement. However, the pulmonary consolidation persisted. The third admission was in November, 1954. The patient had become progressively worse and sputum production had increased. In view of her clinical course a diagnosis of alveolar cell carcinoma was suggested. Examination of the sputum by the Papanicolaou technic was reported as normal, although later review of the smear revealed atypical cells. She was discharged without improvement and died on March 3, 1955.

Autopsy revealed bilateral pleural effusion and an adhesive pleuritis of the right lung. The right lower lobe was black, fibrotic and small but otherwise the lungs were large and bulky. Cut sections revealed that the major portions of both lungs were diffusely consolidated and appeared gelatinous. The bronchi contained foamy mucoid secretions but were free of stenosis, obstruction or tumor. No carcinoma was present elsewhere in the body.

Microscopic examination showed that the alveoli in innumerable areas of both lungs were lined by a tall, columnar, non-ciliated, mucus producing epithelium of a uniform character. Several areas of scarring and organized pneumonia were present in the right lower lobe (Figs. 3 and 4) in which tumor involvement

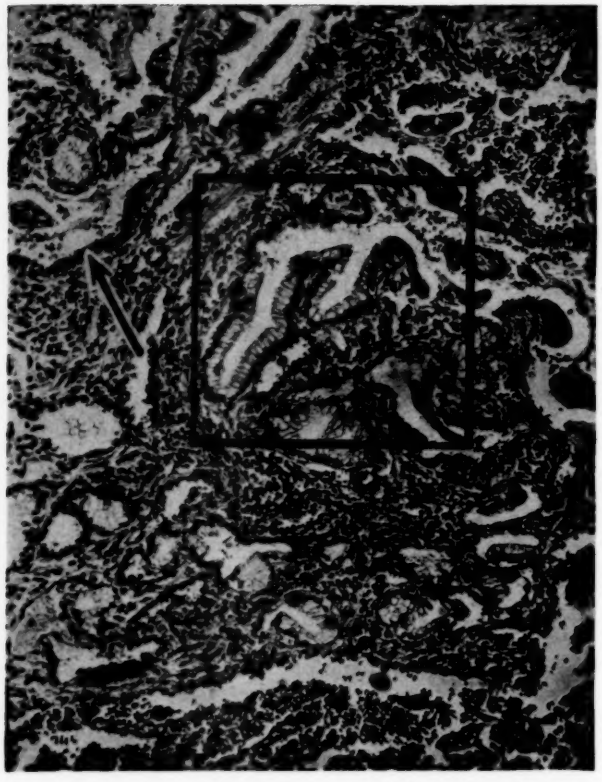

FIG. 5. Case VIII. Adenomatous epithelial hyperplasia in an area of chronic inflammation. Usual low cuboidal epithelium (arrow) in area of chronic inflammation and fibrosis, along with tall columnar cells characteristic of alveolar cell carcinoma.

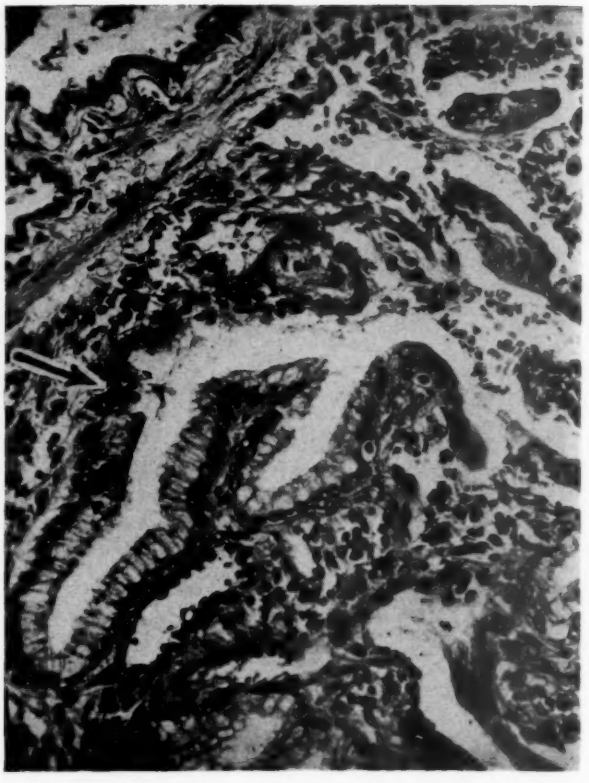

FIG. 6. Same case. High power view of outlined area of Figure 5 showing the transition (arrow) from the ordinarily seen cuboidal epithelium into the tall columnar type of alveolar cell carcinoma.

was most marked. Extrapulmonary metastases were not present.

CASE IV. (A-106-49.) A sixty-six year old white woman was first admitted in May, 1949, with pneumonia of the left lower lobe. Although the patient was treated with penicillin, consolidation still showed on x-ray examination at the time of discharge. She was readmitted two months later, again with pneumonia, and roentgenograms revealed an increase in the area of consolidation as compared with that on the previous admission. Treatment with penicillin and chlortetracycline again failed to bring about complete resolution of the pulmonary infiltration. The last admission was in November, 1949. During the interim the patient had done well subjectively but five days before admission malaise, increased cough, nausea, vomiting and diarrhea had developed. Following admission the patient had extreme respiratory difficulty and died shortly.

The autopsy was limited to an abdominal incision and to removal of the left lower lobe. No extrapulmonary tumor was found on gross examination. The left lower lobe showed multiple white nodules without an endobronchial lesion. On microscopic section the alveoli were lined by tall columnar, non-ciliated, slightly eosinophilic cells with brush borders. There was chronic inflammatory cell infiltration throughout but no proliferation of fibrous tissue. The bronchiolar epithelium appeared intact but moderately hyperplastic.

CASE V. (V. U. H. No. 186,014.) This fifty-five year old white woman was in good health until December, 1949, when she noted the onset of wheezing in the right lower portion of her chest and a cough productive of a small amount of sputum. In January, 1950, the patient had pneumonia. Penicillin therapy was followed by remission of all symptoms except wheezing. Roentgenograms showed a persisting infiltration in the right lower lobe which failed to respond to penicillin or to chlortetracycline. Bronchoscopic examination revealed no abnormalities. In February, 1950, when the patient was admitted to Vanderbilt University Hospital for the first time, chest pain had been present for two months. A right pneumonectomy was performed. In the lower lobe of the excised lung there was an area of diffuse induration 8 cm. in diameter without significant fibrosis or suppuration grossly. Microscopically, the alveoli were lined by tall, columnar, non-mucus secreting cells with nuclear pleomorphism and hyperchromasia. A few mitotic figures were seen but no metastases to the

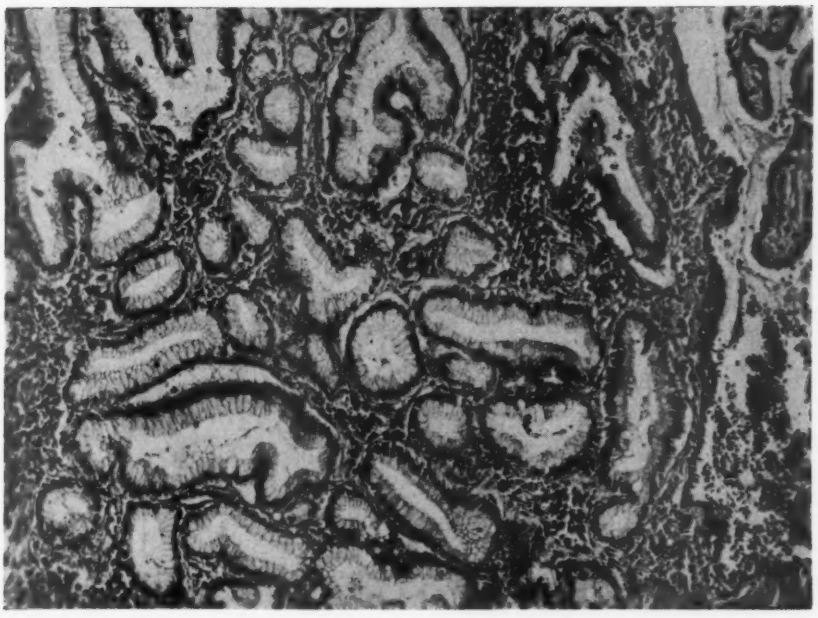

FIG. 7. Case VIII. Classic changes of alveolar cell carcinoma in a contiguous area. Note the thickened septums and the abundant intersitital cellular infiltrate.

regional nodes were demonstrated. Fibrosis, interstitial infiltration of cells and alveolar thickening in this lobe suggested a chronic pneumonitis with origin of the tumor in this area. The right upper and middle lobes were uninvolved and had been removed only after a frozen section following lobectomy had shown carcinoma. The patient did well until 1954 when she began to cough and to complain of dyspnea. Thickening of the left hilar area with a mottled infiltrate appeared on x-ray examination. By April, 1955, the involvement was more diffuse, although it was predominately in the left lower lobe and tumor cells were subsequently demonstrated in the sputum.

CASE VI. (V. U. H. No. 225,163.) A fifty-seven year old white man was admitted to the hospital in July, 1953, because of an asymptomatic "coin" lesion of the right lower lobe found on routine x-ray of the chest. The past history was significant only in that the patient had attacks of "catarrh" as a child and several bouts of "influenza" during the last five years. A right pneumonectomy was performed following wedge resection of a circumscribed mass and frozen section. No lesion was found in the remaining portion of the lung. The bronchi were uninvolved. Microscopic examination showed that the alveolar septums were lined by tall columnar, non-ciliated, non-mucus producing epithelial cells which exhibited considerable variation in size and shape and had hyperchromatic nuclei with frequent mitoses. Physical examination and x-ray in May, 1955, failed to reveal any evidence of recurrence. In this case too the tumor was in an area of chronic inflammation which showed fibrosis and an infiltration of lymphocytes and plasma cells.

CASE VII. (V. U. H. No. 248,382.) This sixty-two year old white woman had a severe case of influenza in 1918. Subsequently she had intermittent episodes of "hacking cough and sinus trouble." In January, 1955, she had pneumonia of the right lower lobe followed by a persistent cough. She was thought to have bronchiectasis, fibrosis and consolidative changes in the right lower lobe. The patient was treated with prolonged antibiotic therapy and postural drainage. She was admitted to Vanderbilt University Hospital on August 19, 1955, for a lobectomy which was performed three days later. The excised right lower lobe revealed no endobronchial disease. The parenchyma was almost gelatinous in appearance. Microscopically, the alveoli were lined with columnar eosinophilic cells with basal nuclei. The cells were uniform in appearance. Considerable fibrosis and septal thickening with an infiltration of chronic inflammatory cells were observed. The alveolar septums in these areas were lined by cuboidal epithelium gradually increasing in height with papillary formations typical of an alveolar cell carcinoma. The postoperative course was uneventful.

CASE VIII. (V. U. H. No. 246,504.) A twenty-three year old white married woman was admitted to the hospital in June, 1955, with a long history of pulmonary disease. At ages four, seven, nine, eleven and thirteen the patient had had pneumonia with cough and associated hemoptysis. About two years prior to admission intermittent, sharp pains in the right upper portion of the chest developed with recurrent attacks of hemoptysis. An x-ray of the chest at that time revealed cavitation in the right upper lobe. Histoplasmin and tuberculin skin tests were positive; the

sputum was negative for tubercle bacilli. Family history was remarkable only in that the patient's grandmother had had tuberculosis. After admission x-rays of the chest revealed a coarse infiltrative process in the right upper lobe, thought to be compatible with fibrotic pulmonary tuberculosis. The patient was given streptomycin, para-aminosalicylic acid and isoniazid in preparation for surgery. On June 28, 1955, lobectomy of the right upper lobe was performed.

Examination of the surgical specimen revealed a dumbbell cystic area (each cavity measuring 3 cm. in diameter) surrounded by a firm fibrous capsule. The pleura was thickened and most of the lobe was fibrotic.

Microscopic examination revealed considerable peribronchial chronic inflammatory reaction with fibrosis and round cell infiltration. The bronchiectatic cyst was lined by flattened cuboidal and pseudostratified columnar ciliated epithelium. The epithelium of the terminal bronchioles was hyperplastic and thrown into papillary folds. In one adjacent area the alveoli were lined by tall columnar non-ciliated slightly eosinophilic cells in the typical pattern of an alveolar cell carcinoma. (Figs. 5 to 7.) Exfoliated cells and mucoid-appearing material were present in the alveolar spaces. Mitoses were not seen. Numerous other sections were taken at random from the adjacent tissue but no similar areas were found. The patient did well and was discharged on the eighth postoperative day.

COMMENTS

Seven cases have been presented in which alveolar cell carcinoma arose or was intimately associated with areas of chronic inflammation in the lung. In four of these cases there was a history of previous lung disease. In four instances the tumor became manifest with or following an acute episode suggestive of inflammatory disease of the lungs. Three patients showed significant scarring grossly and in all seven patients microscopic evidence of chronic inflammation was seen. The eighth case probably represents an example of early alveolar cell carcinoma associated with a long established inflammatory lesion in the lung.

The literature has been reviewed with regard to the relationship of this neoplasm to chronic inflammatory disease. By far the most complete and comprehensive article to date is that of Storey et al.[1] who collected a total of 226 cases and did a statistical analysis of 205. Unfortunately, the presence or absence of inflammatory changes was not noted in the analysis. However, on reviewing the original articles available to us, 121 cases[2–41] were found in which information concerning the past histories or pathologic descriptions is adequate for classification in this respect. (Table 1.) Of these, in 62 per cent there was a history of previous lung disease; in 84 per cent there was gross and/or microscopic evidence of chronic infection or prior inflammatory disease of the lungs with adhesions, fibrosis, bronchiectasis, chronic or organizing pneumonia, lipoid pneumonia and infiltration of chronic inflammatory cells constituting such evidence. Cases of terminal acute pneumonia were not included in the analysis.

TABLE 1
ANALYSIS OF 121 REPORTED CASES OF ALVEOLAR CELL CARCINOMA WITH REGARD TO HISTORY OF PREVIOUS INFECTION AND PATHOLOGIC EVIDENCE OF CHRONIC INFLAMMATION OF THE LUNG

Number of patients reporting presence or absence of previous lung disease	103
Number with positive history	64
Number with negative history	39
Percentage with positive history	62%
Number of patients showing presence or absence of inflammation	88
Number with inflammatory changes	74
Number without inflammatory changes	14
Percentage showing inflammation	84%

Cuboidal epithelial metaplasia of the alveoli has long been known to occur in a wide variety of pulmonary diseases and is encountered fairly frequently in pathologic material. Bell[23] believes that such changes result from a "loss of respiratory function due to the thickening of the interalveolar septa or filling of the alveoli with foreign material. Although Geever, Neubuerger and Davis[43] list tuberculosis as a cause of alveolar epithelialization, in our opinion the association is not frequent. Tuberculous lesions tend either to become isolated from functional pulmonary parenchyma by a fibrous capsule or to extend peripherally with progressive destruction of tissue; in neither instance is there long-standing interstitial inflammation. The fact that alveolar cell carcinoma was not associated with active tuberculosis in any of the cases reviewed is best explained on this basis.

A study by Auerbach, Mims and Goodpasture[42] on pulmonary fibrosis secondary to pneumonia suggests that in recent years there

has been an increasing tendency for organization of exudate and subsequent fibrosis to occur. This was found in 12 per cent of 307 autopsies performed between 1946 and 1950, as compared to 7 per cent and 5 per cent in comparable series of 100 cases each, taken from the years 1940 and 1930, respectively. It was suggested by Auerbach et al. that this changing incidence is due either to an increase in atypical or viral pneumonia or to failure of resolution to occur in bacterial pneumonia. It was postulated that administered antibiotics could lead to alteration in the normal host response to bacterial infection, with failure of resolution and organization of the exudate. The bactericidal agents tend to depress leukocytic infiltration into the area of infection with a resulting deficiency of the enzymes from the polymorphonuclear leukocytes which ordinarily bring about the solution of the fibrinous exudate. If fibrinolysis does not occur organization and fibrosis ensue. The ever increasing use of antibacterial drugs suggests that the latter circumstance is the more important in the increased incidence of organized pneumonitis. In either event Bell's postulate is satisfied. Bell,[23] indeed, goes so far as to state that "there is no good reason to doubt that hyperplasia of the alveolar epithelium may give rise to localized or diffuse adenomatous growths which may form metastases." In this respect Case VIII is especially significant, for it well illustrates the ability of bronchiolar or alveolar epithelium in association with chronic inflammation of lung parenchyma to undergo metaplasia of such a character as to establish the growth pattern of alveolar cell carcinoma. Here, in continuity with the more frequently encountered "epitheliazation of the alveoli," there are the tall columnar cells of pulmonary adenomatosis or alveolar cell carcinoma arising in one focus with no evidence of similar change in adjacent tissue. (Fig. 7.) It is reasonable to believe that this lesion, accidently encountered, represents the incipient stage of alveolar cell carcinoma.

In regard to the increasing incidence of organizing pneumonia and pulmonary fibrosis, it is worthy of note that there has been a remarkable increase in the number of reported cases of alveolar cell carcinoma and apparently this is not a result of more accurate diagnosis. Reviews of autopsied material previously designated simply as pulmonary adenocarcinoma have failed to unearth a significant number of cases of alveolar cell carcinoma when compared to the number which has been recorded in the recent literature. From the original description in 1876 by Malassez[48] until 1942 when Neubuerger and Geever[45] reviewed the literature, only thirty-nine cases of alveolar cell tumors had been reported. Swan[30] in 1949 accepted only twenty-five of these but reported nine of his own cases and collected twenty-six which had appeared since 1941 as well as one case that had not been included by Neubuerger and Geever. Storey[1] in 1953 reported thirty-seven cases and collected 120 others occurring subsequent to Swan's review.

Whether or not the increase in alveolar cell carcinoma is related to the increase in pulmonary fibrosis is a question which cannot be answered finally at this time, as the apparently increasing incidence of pulmonary cancer in general must be taken into consideration. Moreover, whether a focus of chronic inflammation represents the site of origin of these tumors or whether the tumor by its presence predisposes the lung to secondary infection is debatable—although the former seems much more likely from the evidence at hand. It is well known, for example, that neoplasms of bronchogenic origin produce mechanical blockage and thereby predispose the lung to infection. On the other hand, metastatic tumors being peripheral in location and involving the bronchi only secondarily, predispose the lung only slightly if at all to secondary infection. This situation is similar to that present in alveolar cell carcinoma. Certainly in Cases I, II, IV and VIII the pulmonary disease antedated the appearance of the tumor by many years. The possibility of such a relationship between chronic pneumonitis and alveolar cell carcinoma is not in accord with the views of some authors; for example, Good et al.[14] believe that "it is essential for a diagnosis of alveolar cell tumor that the interalveolar septa be thickened only slightly or not at all and that there be little evidence of an inflammatory reaction." Also, Wood and Pierson[6] in one of their cases found "no apparent relationship between the inflammatory foci and the masses of hyperplastic alveolar cells." Our own experience, however, has led us to believe that the two are intimately related, that a cause and effect relationship may exist and that alveolar epithelial or bronchiolar metaplasia secondary to chronic, fibrosing pneumonitis may well represent the precursor of alveolar cell carcinoma. Observation and critical study of future cases will be necessary to substantiate or disprove this thesis.

The question of a multicentric origin would thus be obviated except in rare instances of extensive widespread chronic pulmonary inflammatory disease. Certainly in Case VIII the fact that the change was present in only one area is a strong argument for the unicentric origin of these tumors. Hence it is logical to assume that the tumor arises locally and then spreads to adjacent areas via (1) the pores of Kohn and (2) the bronchi, either aerogenically or by utilization of the copious mucoid secretions as a transportation medium. That tumors might disseminate by this latter method was first suggested by Schuster[46] in 1929 and was reemphasized recently by Hutchison.[16] Although not subject at the present time to experimental proof, this method of spread is not at all contrary to present day concepts of tumor growth. Laryngeal papillomas, for example, having been broken off and aspirated, have been known to implant on mucous membrane further down the bronchial tree.[47] The ability of certain tumors to implant on serous membranes is well known and has been mentioned previously. Statistical evidence strongly suggests that alveolar cell carcinomas do have a unicentric origin. In 26 per cent of the cases analyzed by Storey the tumor was first observed on x-ray examination as a solitary peripheral nodule, and in more than two-thirds of the cases unilateral lesions were observed at the time of the first roentgenographic examination. Such a concept is of more than academic interest, as the multifocal or unifocal nature of their origin is obviously of the utmost importance in treatment.

SUMMARY

1. Seven cases of alveolar cell carcinoma associated with localized chronic inflammatory disease in the lung are presented; an eighth case which may represent the earliest recognizable phase of the disease is also included. The findings in these cases are compared with those in 121 cases taken from the literature.

2. Pathologic evidence is submitted that alveolar cell carcinomas may arise in or are associated with inflammatory foci in the lung. Alveolar cell carcinomas probably arise in a single focus and metastasize first throughout the ipsilateral and then the contralateral lung via mucous sections or aerially.

3. Alveolar epithelial metaplasia may represent a precancerous phase of cellular growth.

4. The increasing incidence of alveolar cell carcinoma parallels and may be related to the reported increased incidence in pulmonary fibrosis.

Acknowledgment. We wish to express appreciation to Dr. Phillip I. Levitan, Dr. David Gotwald and Dr. Hollis E. Johnson, for their assistance in collecting the material.

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The Latex Fixation Test*

I. Application to the Serologic Diagnosis of Rheumatoid Arthritis

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AGGLUTINATION of sensitized sheep erythrocytes by the serums of certain patients with rheumatoid arthritis was first described by Meyer[1] and later by Waaler.[2] In 1948 Rose, Ragan, Pearce and Lipman[3] devised a diagnostic test for rheumatoid arthritis based on this reaction. Heller et al.[4,5] modified this test by the use of a suspension of tannic acid-treated sheep erythrocytes mixed with pooled human plasma fraction II.

The agglutination involved in these tests is really a precipitin reaction which takes place on the surface of a large particle, the erythrocyte. This particle has a complex organic structure, which leads to difficulty in the procedure and uncertainties in interpretation of the reaction. Sheep erythrocytes contain many antigens which react with components of human and animal serums. It has been found that cells from the same animal may not yield a constant titer in repeated tests on the same specimen of serum.[6] Preserved sheep red blood cells, moreover, frequently do not maintain the same degree of agglutinability after prolonged storage.[6,7] Mixed cell suspensions sometimes give end-points in agglutination reactions which are not reproducible. In addition, when sheep erythrocytes are mixed with either antisheep amboceptor or gamma globulin a number of unknown factors are introduced which make the resultant reaction even more complex and difficult to interpret. Serum protein components from many animal species are not easily absorbed by normal erythrocytes.[8]

It was in an effort to overcome some of these difficulties that prior treatment of the erythrocytes with tannic acid was introduced.[4,8] This is laborious, however, and the complexity of the organic and antigenic structure of the erythrocytes is not thereby eliminated. Similar difficulties are encountered with human and other animal erythrocytes.

Preliminary studies had demonstrated that biologically inert polyvinyl toluene latex particles of appropriate and uniform size are suitable for use in serologic studies.[9] When mixed with gamma globulin, the latex particles may be substituted for sheep cells in the investigation of rheumatoid arthritis serums. The behavior of these particles in various electrolytes at different ionic strengths and pH was studied and it was shown that when mixed with gamma globulin these particles acquired new characteristics related to the ampholytic nature of the protein. The pH range between 5.5 and 8.0 must therefore be avoided in any agglutination reaction involving latex particles and gamma globulin since spontaneous agglutination occurs at the isoelectric point of gamma globulin, pH 6.6. It was also determined that optimum agglutination of the gamma globulin-treated particles occurred when the tubes were incubated for two hours at 56°C.

A series of experiments was carried out, using latex particles to determine the conditions under which the interaction between gamma globulin and rheumatoid arthritis serum could best be applied as a diagnostic test for rheumatoid arthritis. These experiments included: (1) various methods of preparation of the latex particles; (2) effect of the addition of a latex particle-gamma globulin suspension to rheumatoid arthritis serum; (3) effect of pH on a mixture of latex particles, gamma globulin and rheumatoid arthritis serum; (4) effect of adding electrolytes to this system; (5) amount of gamma globulin required; (6) order of addition of reagents; (7) effect of temperature; and (8) effect

* From the Arthritis Clinic of the Mount Sinai Hospital, New York, New York. Aided in part by grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, U. S. Public Health Service; the State of New York Chapter, Arthritis and Rheumatism Foundation; and the Fribourg Fund.

of centrifugation on determination of the end-point.

Preparation of Latex Particles. Polyvinyl toluene latex particles of several diameters were tested. The most satisfactory particle size was found to be 0.77 microns; particles 0.82 or 0.98 microns in size were almost as satisfactory. Particles smaller in size than 0.59 microns gave retarded reactions and centrifuged poorly. Polystyrene latex particles with a diameter of 0.81 microns were also satisfactory. The optimum concentration of latex particles in stock solution was found to be such that a 1:100 suspension of the stock solution in distilled water corresponds to a turbidity of 6 per cent light transmission in the Coleman Universal Spectrophotometer. At the time of preparation of the stock suspension, and every two weeks thereafter, the suspension was filtered through Whatman No. 40 filter paper to remove clumped particles. Some spontaneous clumping occurred after several days when the stock solution was prepared with isotonic saline solution alone or with phosphate or acetate buffers diluted with saline solution. The concentration of latex particles in buffer-saline solution added to the test serums was measured at light transmissions of 2, 3, 5, 7, 12, 22 and 32 per cent. The more concentrated suspensions showed precipitation even in control tubes, agglutination with the more dilute suspensions was difficult to read. Optimum concentration of the latex particles was found to be at 6 per cent light transmission.

Effect of Addition of Latex Particle-gamma Globulin Suspension to Rheumatoid Arthritis Serum. One hundred and fifty serums from patients with rheumatoid arthritis and 1,230 control serums were tested by the addition of a mixture of latex particles and gamma globulin. Agglutination occurred in 71.3 per cent of the serums from patients with rheumatoid arthritis and in 2.7 per cent of the control serums. These results will be discussed in detail in a subsequent paper.[10] The test was repeated using a latex particle suspension without gamma globulin. Eleven per cent of rheumatoid serums (those strongly positive by the gamma globulin method) caused agglutination whereas no agglutination occurred in a control group of 250 serums.

Effect of pH. It has already been pointed out that latex particles mixed with gamma globulin, without addition of rheumatoid arthritis serum, precipitate spontaneously between pH 5.5 and 8.0; therefore this pH range is to be avoided in routine work. Phosphate buffer at pH 8, Miller and Golder[11] and veronal buffers ranging from pH 8 to 9 gave occasional non-specific agglutination in control tubes. In buffers consisting of NaOH and Na_2PO_4 (pH range 10.0 to 12.6) and Na_2CO_3 and HCl (pH range 10.1 to 11.4) the reaction is totally inhibited. Borate buffer between pH 8.2 and 9.0 proved to be most suitable for the reaction. The buffer employed for the investigation of rheumatoid arthritis serums was a borate buffer at pH 8.2.

Effect of Adding Electrolytes to the System. No agglutination was observed when distilled water was used as a diluent for the latex-gamma globulin mixture and for the serum being tested. In mixtures of latex particles, borate buffer, gamma globulin and positive rheumatoid serums, the agglutination is relatively weak. The addition of saline solution to the system results in a marked increase in agglutination. Sodium chloride concentrations from 10 to 0.08 per cent in ten intermediate dilutions were tested. The optimum range was found to be between 0.31 and 1.25 per cent. Normal saline solution (0.85 per cent) was chosen as the most appropriate to be added to the borate buffer. Calcium chloride and magnesium chloride did not increase agglutination.

Amount of Gamma Globulin Required. Decreasing amounts of lyophilized gamma globulin (Squibb) ranging from 50,000 gamma per ml. to 0.1 gamma per ml. were added to progressive dilutions of positive serums along with a constant quantity of latex particles. The optimum concentration of antigen varied from 25 gamma to 500 gamma per ml. In an assay of ten positive serums of varying strength it was found that the optimal concentration of gamma globulin was 250 gamma per ml., giving a final dilution in test serums of 125 gamma per ml. Very high concentrations of gamma globulin (over 12,500 gamma per ml.) failed to produce agglutination. Studies are in progress to determine the agglutinating potency of various fractions of gamma globulin and products containing gamma globulin. The effects of antigen and antibody excess are also being studied.

Order of Adding Reagents. Cavelti[12] described for collodion particles a method consisting of coating the particles, washing them and adding a progressive twofold dilution of the serum being tested. When this method was applied to latex particles, non-specific agglutination was observed in the buffer-saline solution control tubes. Goodner[13] described a "collodion fixa-

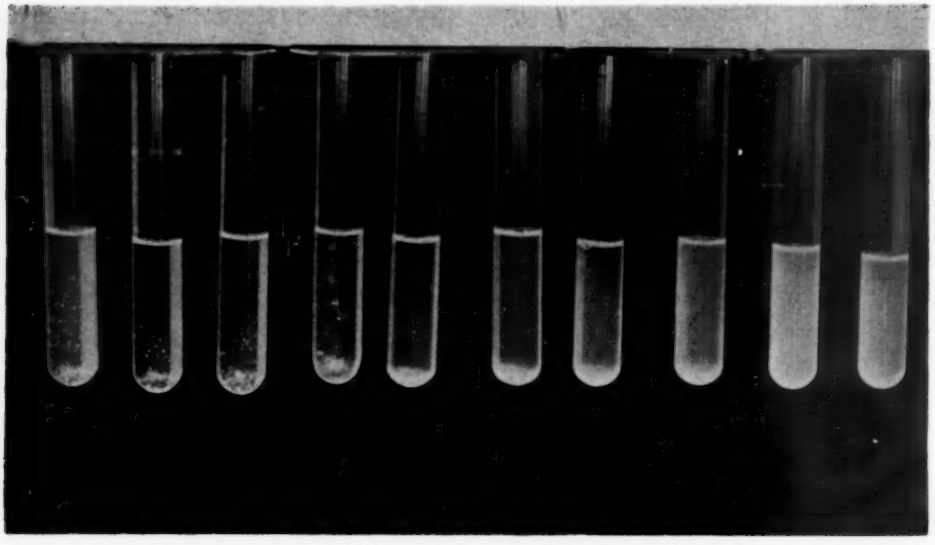

FIG. 1. A typical positive reaction of the latex fixation test (serum of patient with rheumatoid arthritis). From left to right, tubes 1 through 6, progressive dilutions 1:40 to 1:1280, positive; in tubes 7 through 9, negative; tube 10, control.

tion" technic consisting of the simultaneous addition of a mixture of particles and antigen to progressive dilutions of serum. When this technic was applied to latex particles non-specific agglutination did not occur and reproducible results were obtained. For this reason this order of addition of reagents was adopted and the term "latex fixation" was applied to the test herein described.

Effect of Temperature. Fifty positive and fifty negative serums were tested before and after inactivation at 56°C. for twenty minutes. Since no difference was noted it was concluded that inactivation of serum was unnecessary. Eight positive serums with titers ranging from $\frac{1}{80}$ to $\frac{1}{10240}$ were tested in progressive dilutions at 4°C., 37°C. and room temperature for two hours and eighteen hours, 37°C. for two hours followed by refrigeration overnight, and in a water bath at 56°C., 58°C. and 62°C. for two hours. The optimum result was obtained at 56°C. for two hours. The reaction appears to be thermostable.

Effect of Centrifugation on the Latex Fixation Test Method of Reading Results. Following incubation of the test mixtures at 56°C. for two hours, agglutination may be read with difficulty by the naked eye. However, after centrifugation at 2,300 revolutions per minute for three minutes agglutination becomes clearly defined. In strongly positive serums the supernatant is clear (4+), in less positive serums it may be cloudy (1 to 3+). In control and negative serums there is an occasional agglomeration or agglutination of particles after centrifugation but these are readily and smoothly suspended by agitation of the tube. Under unfavorable conditions of testing, such as contamination of the serums or prolonged storage, a coagulum may be observed at the bottom of the tubes, with a turbid supernatant. Occasionally, the control tube will show a fine granulosity and this should be taken into consideration in determining the final titer of the serum tested. The agglutinoscope, strong lens and photocolorimeter were not used.

PROCEDURE FOR LATEX FIXATION TEST FOR THE DIAGNOSIS OF RHEUMATOID ARTHRITIS

Materials

Polystyrene latex particles of a uniform size of 0.81 micron supplied by Dow Chemical Company, Midland, Michigan as a suspension containing 11 per cent solid material. Twenty ml. of water is added to 2 ml. of this suspension and the diluted suspension filtered through Whatman No. 40 filter paper. The resultant stock solution of latex should be sufficiently dense so that when approximately 0.1 ml. of this stock solution is added to 10 ml. of borate buffer the resultant suspension matches a light transmission of 6 per cent in a spectrophotometer at 650 mμ with a red filter. This stock solution may be kept in the refrigerator for several months.

Borate-saline solution buffer at pH 8.2. Fifty cc. of 0.1 M boric acid and 5.9 ml. of 0.1 N NaOH are made up to 100 cc. with water and the pH adjusted to exactly 8.2. 0.85 gm. of sodium chloride is added to each 100 ml. of buffer.

A stock gamma globulin solution of 0.5 per cent in borate buffer at pH 8.2. To 0.5 gm. of lyophilized fraction II add borate buffer in 5 and 10 ml. increments, mixing well each time and saving the supernatant until a total of 100 ml. has been added and the powder is completely dissolved. This stock solution may be stored for weeks under refrigeration.

Method

1. The first tube contains a serum dilution of 1:20 (0.1 cc. of serum and 1.9 cc. of buffer). A progressive twofold dilution of the serum being tested (inactivation and absorption are unnecessary) is prepared with borate-saline solution buffer at pH 8.2 so that each tube contains 1 ml. and a dilution from 1:20 to 1:5120.
2. Each test includes a control tube containing 1 ml. of buffered saline without added serum.
3. To each serum dilution tube containing 1 ml. and to the control tube is added 1 ml. of a mixture containing 1 per cent stock latex and 5 per cent stock gamma globulin in borate buffer. The latex-gamma globulin mixture is prepared by adding 0.1 ml. of stock latex and 0.5 ml. of stock 0.5 per cent gamma globulin to 9.4 ml. of borate buffer. Ten ml. of this mixture is required for each test.
4. The tubes are shaken thoroughly and incubated in a water bath at 56°C. for two hours.
5. The tubes are then centrifuged at 2,300 revolutions per minute for three minutes and the resultant visible agglutination ranging from 0 to 4+ is read with the naked eye. Agglutination in a dilution of 1:20 or greater is considered a positive test. (Fig. 1.)

COMMENTS

In order to obtain comparable titers it is necessary to standardize the conditions of the procedure employed by maintaining constancy of latex concentration, make and source of gamma globulin used, time of incubation and temperature. Borate buffer at pH 8.2 provides the optimal conditions for the test in rheumatoid arthritis. This pH avoids the pH zone around the isoelectric point of gamma globulin where spontaneous agglutination may occur.

Epstein et al.[14] have described a precipitin reaction which occurs with fraction II when added to serums from patients with rheumatoid arthritis. It seems likely that the latex fixation reaction is basically a precipitin reaction occurring at the surface of large particles which then agglutinate.

It is of considerable interest that 11 per cent of rheumatoid serums—those strongly positive by the standard methods—produce agglutination of uncoated latex particles, in the absence of added gamma globulin. This suggests that in these instances the particles become coated with gamma globulin or a fraction thereof from the patient's own serum and are then agglutinated following reaction with another serum fraction. This finding is in accord with Wallis[15] who observed that some rheumatoid serums agglutinated plain collodion particles and suggests the possible presence of autoantibodies in the serum of patients with rheumatoid arthritis. There has been no instance in our studies of precipitation of uncoated particles in normal serums.

In a study of other systems to determine whether the latex fixation technic could be applied to other antigen-antibody reactions, two systems have shown a great affinity for the particles: antipneumococcus rabbit serum type VII and pneumococcus polysaccharide type VII, and anti-C-reactive protein serum and serum from patients with rheumatic fever and allied conditions.

SUMMARY

1. A latex fixation test for the serologic diagnosis of rheumatoid arthritis is described.
2. The principle of the test is similar to that of current technics depending upon the agglutination of erythrocytes but the substitution of biologically inert polyvinyl toluene and polysterene latex particles of uniform size obviates difficulties attendant upon the use of erythrocytes. The test consequently is more simple to perform and to interpret.
3. The latex fixation test must be performed under rigidly standardized conditions in order to obtain reproducible titers.

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The Latex Fixation Test*

II. Results in Rheumatoid Arthritis

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SINCE Cecil, Nicholls and Stainsby[1] reported the agglutination of beta hemolytic streptococci by the serum of certain patients suffering from rheumatoid arthritis, considerable attention has been given to this serologic characteristic. When Rose, Ragan, Pearce and Lipman[2] described a test involving the agglutination of sensitized sheep erythrocytes by the serums of patients with rheumatoid arthritis great impetus was given to serologic investigations in rheumatic disease. Many modifications of this "differential sheep-cell test" have been proposed in an effort to simplify the test, increase its specificity and investigate the "rheumatoid factor" responsible for the agglutination. Svartz and Schlossmann[3] have emphasized the difficulties which arise in comparing results of different investigators working with sheep-cell tests. These difficulties are due to variations in the erythrocytes, variable concentration of the cells, variability of amboceptor, differences in absorption, differences in the time and method of reading of end-points and problems in distinguishing positive from doubtful and negative results. Heller et al.[4] found the amboceptor could be replaced by human plasma fraction II and tannic acid-treated erythrocytes. This observation has led to numerous studies of possible interaction between fractions of gamma globulin and fractions of rheumatoid serums. Epstein et al.[5] noted a direct precipitin reaction between serum from rheumatoid patients and a preparation of human gamma globulin. While this test eliminates the use of erythrocytes, it is technically long and difficult and does not lend itself readily to routine use.

In our search for a biologically inert particle which could replace red blood cells and thus simplify serologic testing in rheumatoid arthritis, we found that polyvinyl toluene or polystyrene latex particles of appropriate and uniform size are suitable carriers of fraction II in the agglutination reactions.[6] Our previous studies dealt with some of the physico-chemical properties of the latex particles and we have described an optimum procedure for utilizing the "latex fixation reaction."[6,7] This test depends upon the addition of latex and gamma globulin mixture to a progressive dilution of the serum to be tested. The present clinical study was undertaken to determine the results of the new test in rheumatoid arthritis, other disease states and in normal control subjects, and to compare the results of the latex test with those of the Rose-Ragan and Heller tests.

CLINICAL MATERIALS AND METHODS

The latex fixation technic has been employed to test 1,380 serums obtained from patients in the wards and clinics of the Mount Sinai Hospital. Normal serums were obtained from the routine Wassermann laboratory. Of the serums tested 150 specimens were from patients with a clinical diagnosis of rheumatoid arthritis, 120 had the submitted diagnosis of osteoarthritis or other non-rheumatoid arthritis. There were 250 serums from patients with rheumatic fever or rheumatic heart disease,† twenty from patients with disseminated lupus erythematosus, eighty specimens from patients who had hyperglobulinemia not associated with rheumatic disease, 560 serums were from patients with other non-arthritic diseases and there were 200 normal control serums.

All these serums were tested with the latex fixation technic.[7] The 150 serums from rheumatoid arthritis patients were also tested by the technics of Rose, Ragan et al.[2] and Heller.[4] The serums of 270 patients

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with non-rheumatoid disease were tested with the Rose-Ragan method and eighty patients without arthritis by the Heller technic. With both of these latter methods, the serum tested was inactivated and absorbed to remove heterophil antibody.

TABLE I
RESULTS OF LATEX FIXATION TEST IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OTHER CONDITIONS

Clinical Groups	Total Cases	Positive	% Total
Rheumatoid arthritis	150	107	71.3
Osteoarthritis and other arthritis	250	5	2
Rheumatic fever and rheumatic heart disease	250	4	1.6
Diseases with hyperglobulinemia	80	4	5
Lupus erythematosus	20	1	5
Other non-arthritic disease	560	17	3
Normal	200	2	1
Total groups	1380	...	.....

RESULTS

The results of the latex test are summarized in Table I. Of the 150 serums from rheumatoid patients, 107 (71.3 per cent) were positive. Positive tests were obtained also from serums in 1 per cent of normal controls, 3 per cent of patients with non-arthritic disease, 5 per cent of patients with hyperglobulinemia, 2 per cent with the diagnosis of osteoarthritis and other non-rheumatoid arthritis, 1.6 per cent of the rheumatic fever—rheumatic heart disease group and 5 per cent of patients (1 to 20) with disseminated lupus. There was an overall incidence of 2.7 per cent positive results in patients who did not have a clinical diagnosis of rheumatoid arthritis.

Table II compares the results of the latex fixation reaction with the sheep-cell agglutination test of Rose, Ragan et al.[2] in 150 patients with rheumatoid arthritis and 270 patients with non-rheumatoid disease. As suggested by Svartz and Schlossman,[3] a sheep-cell agglutination titer of 1:64 or greater was considered positive. Titers of 1:32 were classified as doubtful and those below this titer as negative. Using these final agglutination titers, only 46.6 per cent of the rheumatoid group gave positive sheep-cell reactions as compared with 71.3 per cent with the latex reaction. The control groups gave about the same low percentages of positive results. There were no doubtful latex fixation results, whereas 21.3 per cent of the sheep-cell tests were so classified. In an effort further to clarify the comparison between the latex and sheep-cell tests, the number of positive latex tests within each dilution titer of the sheep-cell reaction was noted. The results are shown in Table III. Seventy serums had sheep-cell agglutination titers of 1:64 or greater, and sixty-five of these also gave positive latex fixation reactions. Of the thirty-two serums classed as doubtful sheep-cell agglutinators (titer of 1:32) fourteen were positive by the latex technic. Of forty-eight negative sheep-cell rheumatoid serums, twenty-eight were positive with the latex test.

Table IV compares results by the latex and Heller methods in the same group of 150 rheumatoid patients and in eighty patients with other diseases. The doubtful group in the Heller test includes both titers of 1:32 and higher titers which exhibited a prozone phenomenon. By the Heller technic 60 per cent of rheumatoid

TABLE II
COMPARISON OF LATEX FIXATION AND SHEEP CELL AGGLUTINATION REACTIONS IN RHEUMATOID ARTHRITIS AND OTHER DISEASES

Material	No. of Cases	Latex Fixation		Sheep Cell Agglutination					
		Positive	%	Positive	%	Doubtful	%	Negative	%
Rheumatoid arthritis	150	107	71.3	70	40.7	32	21.3	48	32.
Other diseases	270	7	2.6	8	2.9	11	4.1	251	92.6

serums were positive and 16 per cent were doubtful. The control percentage of positives was 3 per cent, about the same as with the latex and sheep-cell methods. Of the 16 per cent of doubtful Heller results, 12 per cent gave positive latex results. Of the 24 per cent of serums which were negative to the Heller test, none gave positive latex fixation reactions. In general, the Heller-positive serums gave agglutination in higher titer than the latex-positive serums. These data indicate a close similarity in results with the two technics.

COMMENTS

The latex fixation reaction, as applied to rheumatoid arthritis, has provided us with a relatively simple, rapid and direct method of testing interaction between serum and fraction II. The latex particles, being biologically inert, provide a uniform surface free from organic interference.

In previous agglutination tests a rather large group of patients gave titers classified as doubtful (titer of 1:32) and this may be clinically confusing. The latex fixation test gave no doubtful results in the series tested and there is no necessity for using an arbitrary titer as an end-point. As a result, it may be possible better to categorize the sheep-cell doubtful patients by the use of the latex test. Many rheumatoid patients in whom sheep-cell tests were negative (titer 1:16 or less), were positive when tested with the latex fixation test. This may indicate greater specificity of the latex test but may also be a manifestation of the absence of biologic interference or inhibition associated with the use of untreated erythrocytes. The overall results with latex particles closely parallel those of the Heller test but the latex test eliminates the use of erythrocytes and the difficulties of treatment with tannic acid.

Since the latex reaction involves an interaction between normal plasma gamma globulin and serum, a group of eighty patients with hyperglobulinemia not associated with rheumatoid arthritis was tested. Only four gave a positive result, but this total of 5 per cent was the highest of any non-rheumatoid group. The total is sufficiently low, however, so that it may safely be assumed that elevation of serum globulin alone is not an important factor in producing a positive latex test.

The group of patients with disseminated lupus erythematosus tested was too small to justify any conclusion that the latex reaction is consistently negative in this disease. The very low incidence of positive results in the rheumatic fever–rheumatic heart disease group is striking

TABLE III

NUMBER OF POSITIVE LATEX FIXATION TESTS WITHIN EACH SHEEP CELL AGGLUTINATION TITER GROUP IN RHEUMATOID ARTHRITIS

	No. of Cases	Dilution of Serums									
		0	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Sheep cell agglutination	150	12	10	14	12	32	16	21	17	14	2
	No. of Cases	Positive									
Latex fixation	150	5	4	8	11	14	15	19	15	14	2

TABLE IV

COMPARISON OF LATEX FIXATION TEST WITH SHEEP CELL AGGLUTINATION AND HELLER TEST IN RHEUMATOID ARTHRITIS AND OTHER DISEASES

Material	No. of Cases	Latex Fixation			Sheep Cell Agglutination			Heller Test		
		% Positive	% Doubtful	% Negative	% Positive	% Doubtful	% Negative	% Positive	% Doubtful	% Negative
Rheumatoid arthritis	150	71.3	..	28.7	46.6	21.3	32.	64.	10	26.
Other diseases	80	2.5	..	97.5	2.	1.5	96.5	2.5	2	95.5

and of differential diagnostic import. These results are more impressive when it is considered that many patients in this group had marked elevation of the erythrocyte sedimentation rate and C-reactive protein. In order further to clarify the specificity of the latex test, 120 patients with osteoarthritis and other non-rheumatoid arthritis were tested. The overall incidence of "false positive" results of 2.6 per cent compares satisfactorily with the results obtained with previous agglutination tests.

The results in general, therefore, indicate that the latex fixation reaction may be a valuable aid in the clinical diagnosis of rheumatoid arthritis. The percentage of positive results in patients with rheumatoid arthritis compares favorably with previously described routine tests and the incidence of such results in other diseases is less than 3 per cent. The reagents may be obtained commercially and prepared by any trained laboratory technician. The test itself is easily performed and may be brought to completion in a single morning.

Of considerable interest is the group of 25 to 30 per cent of patients with clinical rheumatoid arthritis in whom latex and other agglutination tests were negative. Further studies with the latex test are necessary to determine whether this is due to the absence of an agglutinating factor or the presence of an inhibiting factor in the serum. Such studies are in progress at present, using fractionation technics in an effort to clarify the mechanisms of both positive and negative results.

CONCLUSIONS

1. A new serologic test for rheumatoid arthritis, the "latex fixation test," has been performed in 150 patients with rheumatoid arthritis and 1,230 patients with other disorders. The latex fixation technic employs standard reagents and may be easily performed in a single morning.

2. The serums of 71.3 per cent of rheumatoid patients and 2.7 per cent of the control groups gave positive agglutination of latex particles mixed with pooled human gamma globulin (fraction II).

3. The results compare favorably with those obtained by the use of the Rose-Ragan sheep-cell method and generally paralleled those obtained with the Heller technic. Doubtful results were eliminated.

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The Clinical Behavior of the Hemagglutination Test for Rheumatoid Arthritis*

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IN 1940 Waaler[1] first observed that a number of serums from patients with rheumatoid arthritis agglutinated in high titer sheep red cells which had been sensitized with rabbit antisheep cell serum. Subsequently other investigators have described serologic tests based on observations similar to those of Waaler.[2] In 1955 the results of a modified hemagglutination test in a small selected group of patients seen in the Arthritis Clinic of the Grace-New Haven Community Hospital were reported.[3] The present paper describes the clinical results with this same modified test in a series of 177 patients with typical or suspected rheumatoid arthritis and in a group of 244 control cases.

MATERIAL AND METHODS

Clinical material for this study was taken from the patients attending the Arthritis Clinic of the Grace-New Haven Community Hospital and the private patients of one of the authors. The majority of these were patients seen at regular intervals of one to three weeks. All were considered to have either definite rheumatoid arthritis or presented a diagnostic problem suggestive of rheumatoid arthritis.

This pool of clinical material was classified in the following manner: the largest group (Group 1), 121 in number, consisted of classic cases of peripheral rheumatoid arthritis, in which all the patients were in Stage 1, 2, 3 or 4, according to the Steinbrocker classification, Stage 1 showing the least involvement and Stage 4 the most. In addition there were included twelve cases of rheumatoid spondylitis (Group 2), six cases of rheumatoid arthritis with psoriasis (Group 3), and eight cases of juvenile rheumatoid arthritis (Group 4). Thirty cases (Group 5) were of undetermined etiology but nevertheless likely to be rheumatoid arthritis.

The test used in this study[4] detects agglutination by the pattern of the settled red cells rather than by agitation. The advantage is primarily in the need for less hemolysin. The serums of most non-rheumatoid patients fail to react and this allows use of the full range of the test.

The components are the usual ones; namely, sheep red cells, rabbit antisheep cell serum and whole human serum which has been inactivated and absorbed. The concentration of hemolysin for sensitization of cells is set by titration in which a normal and a rheumatoid serum serve as standards. The amount of hemolysin to be used in the *clinical* test will place the normal serum below the range of the test while the higher titer of the rheumatoid serum is kept.

RESULTS

Of those with typical peripheral rheumatoid arthritis in Stage 1, 2, 3 or 4, 94 per cent had positive tests. (Table I.) Three of twelve patients with spondylitis, one of six with rheumatoid arthritis and psoriasis, and three of eight with juvenile rheumatoid arthritis gave positive tests. In fourteen of thirty patients (Group 5) with uncertain diagnoses but nevertheless likely to be rheumatoid arthritis, results were positive.

Criteria for the diagnosis of very early rheumatoid arthritis have never been established. Diagnosis of the disease in this stage is often an arbitrary matter. Consequently, it has been with keen interest that we have studied the results of the test in our Stage 1 cases and in Group 5.

* From the Section of Preventive Medicine and the Department of Internal Medicine Yale University School of Medicine and the Arthritis Clinic of the Grace-New Haven Community Hospital. Expenses of this work were defrayed in part by a grant from the United States Public Health Service and by a grant from the Connecticut Chapter of the Arthritis and Rheumatism Foundation. Presented at the Second Interim Scientific Session of the American Rheumatism Association at the National Institutes of Health, Bethesda, Maryland, on December 9, 1955.

Relationship of Results to Extent of Involvement. Stage 1: In all but one of twenty-six patients in Stage 1 results of the test were positive. Of greater interest is the fact that in sixteen of these twenty-six patients tests were performed during the first six months of their disease. Fifteen of these sixteen individuals had positive tests—two as early as two months after onset and two within three months of onset.

TABLE I
RESULTS OF HEMAGGLUTINATION TESTS (177 PATIENTS)

		Number of Cases	Positive Tests	Per cent Positive
Group 1	Classical peripheral rheumatoid arthritis 4 Stages	121	114	94
	Stage 1 26	...	25	96
	Stage 1 16 (Illness less than 6 months)	...	15	93
Group 2	Rheumatoid spondylitis	12	3	25
Group 3	Rheumatoid arthritis with psoriasis	6	1	16
Group 4	Juvenile arthritis	8	3	37
Group 5	Possible rheumatoid arthritis	30	14	46

Stage 2, 3 and 4: Eighty-nine of the ninety-five individuals in Stage 2, 3 and 4 had positive hemagglutination tests. Lack of objective and laboratory signs of inflammation and the stability of the disease were characteristic of the six with negative results.

Group 5: Among Group 5 the test was positive in fourteen of thirty patients. (Table II.) Seven of the fourteen individuals were women who probably had mixed degenerative and rheumatoid arthritis, and two were individuals in whom the diagnosis was either rheumatoid arthritis or scleroderma. In two women in whom the diagnosis most likely was rheumatoid arthritis or disseminated lupus and whose tests were positive, lupus later developed with positive L.E. preparations. In one, the hemagglutination test became negative after the L.E. preparation became positive. In the other, both tests were positive. The remaining three include a case of monarticular disease, an individual in whom the differential diagnosis seemed to lie between rheumatoid and gonococcal arthritis, and a patient with palindromic rheumatism.

TABLE II
POSITIVE TESTS AMONG 30 PATIENTS WITH POSSIBLE RHEUMATOID ARTHRITIS

Degenerative and possible rheumatoid arthritis	7
Rheumatoid arthritis or scleroderma	2
Rheumatoid arthritis or lupus	2
Monarticular arthritis	1
Rheumatoid arthritis or gonococcal arthritis	1
Palindromic rheumatism	1
Total	14

TABLE III
NEGATIVE TESTS AMONG 30 PATIENTS WITH POSSIBLE RHEUMATOID ARTHRITIS

Postpartum arthralgia	5
Intermittent hydrarthrosis	2
Monarticular arthritis	2
Arthralgia—some soft tissue changes negative X-rays	4
Rheumatic fever or rheumatoid arthritis	3
Total	16

Of equal interest are those whose tests were negative. (Table III.) Five women in their thirties had histories characterized by intermittent joint pain and stiffness over periods of one to seven years, which improved during pregnancies and grew worse immediately afterwards. On some occasions their joints appeared to be swollen and hot but never for any protracted period of time. Erythrocyte sedimentation rates were normal and roentgenologic studies were negative or equivocal. Two patients had intermittent hydrarthrosis. The mother of one of these has typical rheumatoid arthritis. Two had monarticular disease, one of whom had had a previous uveitis. Four individuals had complaints characterized by arthralgia with minimal soft tissue changes in the fingers but negative roentgenograms. Fatigue was prominent but sedimentation rates were normal. Three individuals had valvular heart disease of long standing, normal antistreptolysin titers, and intermittent joint pains often associated with some soft tissue changes.

Relation of Titer to Disease Activity. Whether the titer of the hemagglutination test varies

directly with the activity of the disease is a controversial subject. In our early studies[3] it was felt that the titer fell appreciably as the disease became less active in what we called the "remission" type case. In the "chronic" case, this change did not take place. Among the 121 patients with typical peripheral rheumatoid arthritis reported in this paper a distinct titer change was noted only in those who had dramatic Grade 1 clinical remissions. This change occurred in thirteen of fifteen such patients. The test in ten of the thirteen eventually became negative.

These results bear out our beliefs stated in the earlier studies, namely, that it is only in the patient who has a dramatic Grade 1 remission that there is a distinct fall in titer.

Controls. The controls in this study can hardly be called such in the strict sense of the word because approximately half of the 244 were a selected group, selected to give a figure for the specificity of the test in situations which should be quite demanding. At the same time they were to define the clinical illnesses which would be most apt to place stress on the test. These considerations suggested the study of serums which arrived at the Streptococcus Laboratory. They are known to be from patients with "collagen disease" or bizarre and febrile illnesses. It was our belief that at least 100 adult serums should be used. Such selection resulted in one control group that was quite comparable in age and sex to those in the rheumatoid series.

Table IV lists the illnesses found in the group of 121 patients whose serums had been sent to the Streptococcus Laboratory. Those with rheumatic heart disease are most common and were usually admitted for valvulotomy. Febrile diseases are those characterized by unexplained periodic attacks of fever, chills and malaise. Other illnesses include single examples of entities such as infectious hepatitis, measles and infectious mononucleosis.

Two additional control groups included in the study consisted of ninety patients whose serums were received at the Serology Laboratory and thirty-three obstetric patients. There was a wider variety of illness among those whose serums were sent to the Serology Laboratory and "collagen diseases" were much less in evidence. Pregnancy received attention since health and illness rather merge in this condition, and remissions in rheumatoid arthritis occur in its course. The three groups offered the opportunity for accurate diagnosis and for clinical and serologic follow-up. Use of patients rather than well individuals for general appraisal of the specificity of the test is empirical.

The control groups with results of the tests are tabulated separately for completeness and not for comparison since differences in the incidence of positive tests may be due to chance alone. Among the total of 244 patients, 11 or 4.5 per cent had positive tests. Five per cent seems both an accurate and stringent enough figure for the apparent non-specificity of the hemagglutination (sensitized sheep cell) test in usual medical practice.

TABLE IV
POSITIVE HEMAGGLUTINATION TESTS IN CONTROL GROUPS

Clinical Impression	Number of Cases	Positive Tests
Serums from streptococcus laboratory...		
Active rheumatic fever	2	..
Rheumatic heart disease	54	3
Subacute B. endocarditis (R.H.D.)	2	..
Congenital heart disease	3	..
Acute benign pericarditis	3	..
Lupus and probable lupus	4	..
Nephritis	5	..
Diabetes with infection	6	1
Febrile diseases	11	4
Other illnesses	31	..
	121	8
Serums from serology laboratory	90	3
Serums from obstetrical patients	33	0
Total	244	11 (4.5 %)

SUMMARY

In summary several points stand out.

1. The fact that the hemagglutination test is positive in a much smaller percentage of individuals with rheumatoid spondylitis, juvenile rheumatoid arthritis and rheumatoid arthritis with psoriasis remains unexplained.

2. In our hands the titer of the test seems to show distinct changes only in patients (thirteen of fifteen) with dramatic Grade 1 clinical remissions. The test even became negative in ten of the thirteen individuals with such dramatic changes.

3. Among the controls the test was positive chiefly in those with related collagen disease or in individuals whose diagnosis was not determined.

4. The sensitivity of the test in early atypical cases has not yet been proved, chiefly because of the lack of diagnostic criteria. Its sensitivity in typical early or advanced peripheral rheumatoid arthritis is clearly shown, even among individuals with a illness of less than six months.

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On the Biosynthesis of Uric Acid from Glycine-N^{15} in Primary and Secondary Polycythemia*

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It has long been known that distinct hyperuricemia, with or without accompanying hyperuricosuria, may occur in the leukemias (notably the chronic granulocytic and acute leukemias), the myeloproliferative diseases (notably myeloid metaplasia and polycythemia vera), occasionally also in secondary polycythemia, the chronic hemolytic anemias, pernicious anemia; and in conjunction with the lymphomas, multiple myeloma and a variety of other disorders presumably involving augmented turnover of nucleic acids. The present study is concerned with the genesis of hyperuricemia and hyperuricosuria specifically in the polycythemias; however, the general implications of the results may well apply more broadly to the whole category of these disorders.

Erickson's brief case report [*1*] in 1917 includes what is apparently the first mention of hyperuricemia in polycythemia vera, and it was Maude E. Abbott, more celebrated in connection with her studies of congenital heart disease, who made the observation. This finding was confirmed in a detailed study of a case of polycythemia vera by Isaacs [*2*], by the discovery of excessive endogenous uric acid excretion in a case of primary polycythemia by Shelbourne and Hanzal [*3*], and by reports calling attention to the association of erythremia (polycythemia and/or myeloid metaplasia), hyperuricemia and gout [*4–11*]. Failures to find hyperuricemia or hyperuricosuria in patients with polycythemia vera also were recorded [*12–14*], however, so that Reznikoff [*15*], reviewing the status in 1941, was constrained to leave the matter in doubt. Subsequent experience, however, leaves no question that hyperuricemia and hyperuricosuria occur in a substantial proportion of cases of polycythemia vera [*16–21*], notably those entering into or in the phase of myeloid metaplasia [*11*], and occasionally also in cases of secondary polycythemia [*22*]. At the Mount Sinai Hospital [*23*], approximately one-third of ninety-three patients with polycythemia, chiefly polycythemia vera, were found to have serum uric acid levels in excess of 6.5 mg. per cent (normal maximum in adult men by the method employed, about 6.0 mg. per cent).

In 1923 Isaacs [*2*] suggested that in polycythemia vera "increased uric acid in the blood, in the absence of renal involvement, may have its origin in the liberated nuclear material formed in the increased production of red cells, from the extrusion or dissolution of the nuclear material of the normoblasts at their place of origin." This hypothesis corresponds in principle to the prevailing presumptions as to the origin of hyperuricemia, from nuclear disintegration, in the leukemias and analogous disorders involving augmented turnover of nucleic acids. Overproduction of uric acid is implied in each instance, by pathways representing what is essentially an exaggeration of normal processes of formation of endogenous uric acid—an end-product of the catabolism of the purine components of cellular nucleic acids.

Isaacs' concept of the origin of hyperuricemia in polycythemia vera, which has won general acceptance [*6,8,16–21*], is supported by studies indicating enhanced uric acid production ac-

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companying erythrogenesis in recovery from experimentally induced anemia [*24,25*] and in response to liver therapy in pernicious anemia [*26–28*]. It has not, however, been subjected to examination by the more direct means afforded by the incorporation into nucleic acids and uric acid of isotope-labeled precursors such as glycine. This method of analysis would appear to be particularly desirable in view of the discovery that uric acid is synthesized in the body from simple carbon and nitrogen precursors [*29–31*], not only by way of nucleic acids but also by independent, more direct pathways. These immediate avenues play an important role in uric acid biosynthesis in normal man [*32,33*] and are implicated in the overproduction of uric acid in primary gout [*33–35*].

The rate and magnitude of glycine-N^{15} incorporation into urinary uric acid have been determined in a case of secondary polycythemia [*22*] and, by Laster and Muller [*36*], in a case of agnogenic myeloid metaplasia; the results will be discussed presently. The subjects of the glycine-N^{15} feeding experiment herein recorded were two patients with primary polycythemia and one with polycythemia secondary to congenital heart disease. To round out the data, the findings in the previously studied patient with secondary polycythemia [*22*] also were added to the analysis. One of these patients with primary polycythemia and one with secondary polycythemia had associated overt secondary gout, the other two patients did not.

The experiment was designed with the following objectives in mind: (1) to determine whether or not the hyperuricemia and hyperuricosuria occurring in the polycythemias are due to overproduction of uric acid, as would seem altogether likely, and to throw light upon the major metabolic pathways involved in uric acid biosynthesis in the polycythemias; (2) to compare the rate and magnitude of uric acid biosynthesis in primary polycythemia with secondary polycythemia, as an indication of similar or dissimilar metabolic pathways; (3) to determine whether or not the presence or absence of overt secondary gout can be correlated with any differences in purine synthesis in the polycythemias; (4) to determine whether or not the spectrum of endogenous purines (other than uric acid) and methylpurines present in the urine of normal human subjects [*37–41*] is qualitatively or quantitatively altered in the polycythemias, and to measure the degree and rate of glycine-N^{15} incorporation into these metabolites.

METHODS

The patients were hospitalized for study. They were maintained on a low purine, low fat diet of 1,700 to 1,800 calories per day containing approximately 60 gm. protein which was derived chiefly from cheese, eggs, milk and bread (no meat, fish, fowl or legumes). After a week on this diet, three twenty-four-hour urine collections were made for determinations in the control period. At the beginning of the experimental period each subject was given glycine-N^{15} (60 atom per cent excess N^{15}) in a single oral dose of 100 mg./Kg. body weight. Daily twenty-four-hour urine collections were then made for twenty-one successive days, and on specified days for varying periods thereafter.

Glycine-N^{15} was synthesized from potassium phthalimide containing approximately 60 atom per cent excess N^{15} by the method of Schoenheimer and Ratner [*42*]. The urine was analyzed for total nitrogen (micro-Kjeldahl method), urea nitrogen [*43*], uric acid [*44*] and creatinine [*45*]. Uric acid was isolated and purified by the method of Geren et al. [*46*] and prepared for N^{15} analysis by the method of Sprinson and Rittenberg [*47*]. Purines other than uric acid were isolated from the urine by absorption on a Dowex-50 column, elution with ammonia, precipitation with acid silver nitrate, and separation on an ion exchange column [*40,41*]. Urea was isolated from the urine as the dixanthydrol derivative [*48*]. N^{15} was determined in a 60° mass spectrometer, using the ammonia derived from each component by Kjeldahl digestion [*47*].

CASE REPORTS

L. M. (M.S.H. 34376), a white man, was fifty-two years old in 1954 when the isotope studies to be described were carried out. He had polycythemia vera and there were indications that he was entering into the phase of myeloid metaplasia. There was associated secondary gout but there was no familial history of gout.

He first consulted a physician in 1937 because of recurrent headaches and was found to have hypertension and polycythemia. There were no evidences of congenital heart disease or of pulmonary disorders to which the polycythemia might be ascribed. The polycythemia was treated over the years by repeated phlebotomy and courses of P^{32} and triethylene melamine; the last treatment was given in October, 1953. Apart from the headaches, cyanosis, some lassitude and, latterly, an increasing sense of fullness in the abdomen, he had been free of complaints referable to polycythemia. He gave no history suggestive of thromboses, hemorrhage or congestive heart failure.

In April, 1953, sudden painful swelling of the right wrist developed which was attributed to sprain; an x-ray film was negative. The attack subsided spontaneously in about two weeks but swelling and some pain persisted. There was also mild discomfort at the base of the great toes. In August, 1953, there was sudden onset of olecranon bursitis on the right, three weeks later also on the left. At this point the diagnosis of gout was made and he was referred to the Mount Sinai Hospital for treatment of acute gouty arthritis. In addition to the evidences of polycythemia vera (to be described) and hypertension (blood pressure 185/105) there was persistent swelling of the right wrist, fluid and tophaceous deposits in both olecranon bursae, and some swelling and tenderness at the base of the great toes. The serum uric acid was 13.3 mg. per cent. The therapeutic response to colchicine was excellent. He was maintained on a regimen of daily colchicine prophylaxis, with satisfactory control of the acute attacks.

A course of triethylene melamine was then administered. After receiving two doses, each 5 mg., over a three-day period, the serum uric acid rose to 17.4 mg. per cent and the urinary uric acid from 750 to 1,200 mg. per twenty-four hours. Subsequently, despite continued administration of triethylene melamine (four doses, each 5 mg.) the serum uric acid leveled off at 10 mg. per cent and the urinary uric acid excretion returned to premedication values.

In March, 1954, the patient was readmitted to the Mount Sinai Hospital for study. He was a small man, thin, plethoric and markedly cyanotic, but in no acute distress. The vital signs were normal, the blood pressure was 170/85. There was no lymphadenopathy in the neck or elsewhere. The conjunctivas were suffused, the veins of the fundi were full. The lungs were clear to percussion and auscultation. The heart was somewhat enlarged to the left and a faint blowing systolic murmur was audible at the apex with transmission to the left axilla. The second aortic sound was accentuated. The most striking finding on physical examination was a visible protrusion of the left side of the abdomen due to an enormous, hard spleen which extended well below and to the right of the umbilicus. The liver edge, which also was firm and obtuse, was easily palpable three fingersbreadth below the costal margin. Varicosities were present in the veins of both legs, and old pigmented areas of the skin extended above the ankles. The only indications of tophaceous deposit were in the olecranon bursae, notably on the right. X-ray films, however, revealed characteristic punched-out areas at the metatarsophalangeal joints of both great toes.

The red blood cell count at the time of admission was 6.7 million per cu. mm., hemoglobin 18.0 gm. per cent, hematocrit 64 per cent. The white blood cell count was 14,650 per cu. mm., with a normal distribution of cells and no abnormal forms. Platelets were abundant. The urine was normal. Serum uric acid determinations on several occasions varied from 12.1 to 14.4 mg. per cent (blood urea nitrogen 17 mg. per cent).

Upon hospitalization the patient, who had been maintained on a low purine, restricted protein diet for some time, was given an 1,800 calorie diet which was low in purine, meat-free, and contained 60 gm. protein and 60 gm. fat per day. Colchicine prophylaxis was continued. On the morning of March 4, he was given a single oral dose of 5.35 gm. glycine-N^{15} (100 mg./Kg. body weight). Complete collections of urine were made daily for twenty-one days thereafter, and on selected days for forty-two days after the administration of isotope, while the patient continued on the same diet.

Of interest is his subsequent course: progressive decline in erythrogenesis and increase in early granulocytic cells, typical of the evolution of polycythemia vera in its course as a myeloproliferative disorder toward the phase of myeloid metaplasia. In May, 1956, the red blood cell count was 3.65 million per cu. mm., hemoglobin 9.6 gm. per cent, hematocrit 34 per cent. The white blood cell count was 27,000 per cu. mm., with 7 per cent myelocytes and metamyelocytes.

J. B. (M.S.H. 12893), a white man, was sixty-four years old in 1955 when the isotope studies were made. He had polycythemia vera complicated by duodenal ulcer. There were no clinical manifestations of gout.

Since 1943, the patient had been subject to periods of epigastric distress which were relieved by food, and he had noted tarry stools on occasion. In 1947, the diagnosis of polycythemia vera was first established and he was treated in subsequent years by phlebotomy at intervals and courses of P^{32}. In August, 1952, he suffered the first of a series of gastrointestinal hemorrhages severe enough to require hospitalization. At that time the spleen was palpable two fingersbreadth below the costal margin, the liver was not enlarged. A duodenal ulcer was apparent in the x-ray film. Sternal marrow smear showed marked erythroid hyperplasia. Recurrent gastrointestinal hemorrhages in July and November, 1953, and January, 1954, required subsequent hospital admissions.

The patient was readmitted to the Mount Sinai Hospital in January, 1955, for study. He was described as well nourished, of ruddy complexion but not overtly cyanotic, and in no distress. The vital signs were normal. The blood pressure was 135/80. No abnormalities of the lungs or heart were noted, except for a soft, blowing systolic murmur at the cardiac apex. The liver edge could be palpated two fingersbreadth below the costal margin on deep inspiration; the border was sharp, of normal consistency and nontender. The tip of the spleen descended four fingersbreadth below the left costal margin and was unduly firm and blunt. There were no abnormalities of the extremities; no tophaceous deposits were in evidence.

Roentgenograms showed no indication of uric acid accumulations in or around predisposed joints.

The red blood cell count was 7.85 million per cu. mm., hemoglobin 15.5 gm. per cent, hematocrit 57 per cent, white blood cell count 15,700 per cu. mm. with a normal differential pattern. Sternal marrow smear was normal. Serum uric acid determinations gave values varying from 8.2 to 8.7 mg. per cent while the patient was on a restricted diet. The urinary uric acid excretion averaged 525 mg. per twenty-four hours.

The patient was given a diet which was low in purines, protein (60 gm./day) and fat, and contained 1,800 calories. One week later, on January 10, 1955, he received a single oral dose of 6.37 gm. of glycine-N^{15} (100 mg./Kg. body weight). Twenty-four hour collections of urine were made daily for twenty-one days, then on selected days over a period of about three months.

G. D. (M.S.H. 7119), a white man, was 23 years old in 1955 when last admitted to The Mount Sinai Hospital. He had marked polycythemia secondary to a congenital cardiac lesion, without any indications of associated acute or chronic gouty arthritis.

He had had no cyanosis or dyspnea at birth or in early childhood; he had not assumed the squatting or equivalent positions, and was not obliged to restrict activity as a child. There was no history of rheumatic fever. A heart murmur, not further described, was first noted when the patient was two years old. When he was nine, he first experienced dyspnea and fatiguability on climbing three flights of stairs but could still negotiate two flights comfortably. At eleven, limitation of activity was more marked and some cyanosis of the nail beds was noted. Exertional dyspnea progressed and in 1947, when he was fifteen, he entered the Johns Hopkins Hospital for study. The vital signs were normal. The heart was not enlarged by percussion. A loud, rough systolic murmur was audible over the entire precordium, maximally along the left sternal border at the fourth interspace. The second sound at the apex was accentuated and snapping; no diastolic murmur was heard. The pulmonic second sound was accentuated and louder than the aortic second sound. The lungs were clear. The liver and spleen were not demonstrably enlarged. There was slight clubbing and cyanosis of the fingers. The femoral artery pulsations were full on both sides. Fluoroscopic examination showed the heart to be of normal size; there was a conspicuous pulmonary conus, large pulmonary arteries were visible especially on the right, the vascular markings were prominent throughout, the aorta could be clearly delineated on the left. Electrocardiogram showed right axis deviation with right ventricular hypertrophy, and a slight increase in intraventricular conduction time. The red blood cell count was 5.3 million per cu. mm., hemoglobin 14 gm. per cent, hematocrit 43.5 per cent.

Catheterization studies were recommended but were deferred by the patient until 1950 when he reentered the Johns Hopkins Hospital. In the interim, exertional dyspnea, fatiguability, cyanosis and clubbing of the fingers had increased. Progression in these particulars was confirmed by examination which otherwise disclosed no significant change since 1947. However, the red blood cell count was now 7.2 million per cu. mm., hemoglobin 21 gm. per cent hematocrit 62 per cent. On cardiac catheterization, moderate hypertension in the right ventricle and pulmonary bed (74/32 mm. Hg) was noted. Arterial blood oxygen saturation was markedly reduced (79.5 per cent). A left-to-right shunt of moderate proportions was inferred. These findings, together with the apparent small size of the ventricles on fluoroscopy, led to the diagnosis of a single ventricle, pulmonary hypertension, no transposition of the great vessels. The patient was discharged.

His subsequent course was dominated by the consequences of his marked secondary polycythemia. In April, 1952, and intermittently thereafter, profuse hemoptyses occurred. In September, 1952, he suffered what appears to have been a cerebral thrombosis, manifested by transient aphasia and persistent numbness of the right arm and right side of the body. In January, 1953, there was another occlusive episode, this time of sudden onset of numbness and tingling in both lower extremities, followed by staggering and collapse. Thereafter he noted intermittent claudication which severely limited his activity.

When first admitted to The Mount Sinai Hospital in March, 1953, he presented as a strikingly cyanotic but fairly well developed and well nourished young man, in no acute distress at rest. The physical examination was as previously noted in 1947 except that cyanosis and clubbing were more pronounced, and there was now evidence of impairment of blood flow to both lower extremities, presumed to be due to a thrombosis at or near the bifurcation of the aorta. Fluoroscopy and roentgenograms again indicated no enlargement of the heart in its transverse diameter but the apex was rounded and raised above the diaphragm; in the oblique and lateral views there appeared to be some enlargement of the right ventricle. The pulmonary artery segment was prominent, the pulmonary vascular markings were exaggerated, no hilar dance was noted. The aortic arch and descending aorta were in the normal position. No enlargement of the left atrium was apparent after barium swallow. Electrocardiogram again showed right axis deviation. Cardiac catheterization revealed a marked increase in the pressures in the right ventricle (160/0 mm. Hg) and pulmonary arteries (160/100 mm. Hg). The oxygen content of blood samples obtained from the right ventricle and pulmonary arteries was consistently higher than from the right atrium, indicating a left-to-right shunt into the right ventricle. The Evans blue dye circulation time (right ventricle to ear) was

rapid but not unequivocally indicative of an overriding aorta. The findings were considered to be consistent with the diagnosis of Eisenmenger complex; corroboration by angiocardiography was sought but the results were inconclusive. The presence of an obstructive lesion at the lower end of the aorta was confirmed by the finding of markedly reduced pressure in the femoral as compared with the brachial arteries.

The red blood cell count on this admission was 8.0 million per cu. mm., hemoglobin 20.3 gm. per cent, hematocrit 73 per cent, white blood cells 9,400 per cu. mm. with a normal differential cell count. Several phlebotomies were performed and the patient was discharged. His hemoptyses subsequently recurred, there was another episode suggestive of cerebral thrombosis with transient paresis of the left arm, his exercise tolerance was further reduced by intermittent claudication.

His last admission to the Mount Sinai Hospital was in January, 1955. The findings were as previously noted. The red blood cell count was 7.8 million per cu. mm., hemoglobin 20.7 gm. per cent, hematocrit 71 per cent, white blood cells 6,400 per cu. mm., with a normal differential cell count. The serum iron was 119 gamma per cent. Sternal marrow puncture showed erythroid hyperplasia compatible with (secondary) polycythemia. The blood urea nitrogen was 11 mg. per cent. The serum uric acid was 8.3 mg. per cent while the patient was on the regular hospital diet, and 6.5 to 6.8 mg. per cent while the patient was maintained on a low purine, meat-free diet providing 60 gm. protein, 50 gm. fat and 250 gm. carbohydrate per day.

On February 22, 1955, the patient was given a single oral dose of 4.95 gm. (100 mg./Kg. body weight) glycine-N^{15}. Twenty-four hour urine collections were made daily for twenty-one days, then at intermittent intervals for ninety-two days.

E. F. (M.S.H. 642,387), a white man, was fifty years old in 1952 when isotope studies were made. He had marked polycythemia secondary to a congenital cardiac lesion, and a long history of recurrent acute gouty arthritis and tophaceous gout. A detailed case report appears elsewhere [*22*].

In brief, the patient was a "blue baby" at birth and deep cyanosis, with marked clubbing of the extremities, persisted throughout life. The precise nature of the cardiac anomaly and position of the right-to-left shunt subsequently established by cardiac catheterization was never determined but the case fell in the category of situs inversus of the abdominal viscera with levocardia, which may be associated with complex malformations [*49*].

Polycythemia was noted at the age of twenty which, in later years, led to thrombotic episodes in the brain and elsewhere. The first attack of acute gouty arthritis occurred at the age of thirty-five, thereafter attacks occurred at increasingly frequent intervals until hardly a month passed without one. These attacks responded to colchicine. Tophi were noted when the patient was thirty-seven and subsequently increased so rapidly in size and number that disability due to chronic gouty arthritis became very marked. There was no familial history of gout.

The isotope studies were initiated on June 14, 1952, when he was given a single oral dose of 6.75 gm. glycine-N^{15} (100 mg./Kg. body weight). At that time the red blood cell count was 7.7 million per cu. mm., hemoglobin 22.8 gm. per cent, hematocrit 76 per cent, white blood cell count 5,750 per cu. mm., with a normal differential distribution. The serum uric acid was 10.2 mg. per cent (blood urea nitrogen 22 mg. per cent) and the urinary uric acid excretion 317 mg. per twenty-four hours while the patient was on a low purine, meat-free diet restricted in protein (67 gm./day) and fat.

The essential data relating to these cases are summarized in Table I.

RESULTS

Total Urinary Excretion of N^{15} Atom Excess. Time curves of urinary excretion of N^{15} in all four cases of polycythemia corresponded with the normal [*33,34*]. The greatest concentration of N^{15} occurred in the first twenty-four hour urine collection (1.164 atom per cent excess N^{15} in Case L. M., 2.407 in Case J. B., 2.393 in Case G. D., 2.417 in Case E. F.; representing 23.4, 29.0, 40.1 and 12.6 per cent, respectively, of the ingested atom excess N^{15}). These concentrations declined precipitously for the first four or five days, then very slowly thereafter for weeks. On the tenth day the atom per cent excess N^{15} excretion per twenty-four hours was 0.069 in Case L. M., 0.089 in Case J. B., 0.041 in Case G. D., 0.088 in Case E. F. The respective cumulative total N^{15} excretion, in terms of per cent of ingested glycine-N^{15}, was 51.1, 59.4, 63.9 and 32.3 per cent at the end of ten days, and 65.9, 69.1 and 68.3 per cent (in Cases L. M., J. B. and G. D.) at the end of twenty-one days.

The urinary urea-N^{15} excretion closely paralleled the total urinary N^{15} excretion.

Rate of Incorporation of Glycine-N^{15} into Urinary Uric Acid in Polycythemia Vera. (Table II.) As indicated in Figure 1, the rate curves of glycine-N^{15} incorporation into uric acid for the two patients with polycythemia vera (Cases L. M. and J. B.) were virtually identical; and, interestingly enough, coincided with that of a case of agnogenic myeloid metaplasia described by Laster and Muller [*36*]. Unlike the normal subject (E. B.), whose peak incorporation of about

TABLE 1
SYNOPSIS OF CASE HISTORIES

Name, Age, Weight (kg.)	Type of Polycythemia	Hemogram				Uric Acid		Clinical Résumé
		Red Blood Cells (million/cu. mm.)	Hemoglobin (gm. %)	Packed Red Cell Volume (%)	White Blood Cells (per cu. mm.)	Serum (mg. %)	Urine (mg./day)	
L. M. 52 53.5	Primary	6.7	18.0	64	14,650	12.1–14.4	565	Polycythemia vera of 17 years known duration; myeloid metaplasia; clinical manifestations of (secondary) gout for 1 year; early tophaceous deposits
J. B. 65 63.7	Primary	7.8	15.5	57	15,700	8.2– 8.7	560	Polycythemia vera of 8 years known duration complicated by duodenal ulcer; ? entering the phase of myeloid metaplasia; hyperuricemia but no clinical manifestations of gout, no tophi
G. D. 23 49.5	Secondary	7.8	20.7	71	6,400	6.5– 6.8	329	Polycythemia secondary to congenital heart anomaly; equivocal hyperuricemia, no clinical manifestations of gout, no tophi
E. F. 50 67.5	Secondary	7.7	22.8	76	5,750	9.8–11.2	343	Polycythemia secondary to congenital heart anomaly; hyperuricemia; clinical manifestations of (secondary) gout for 15 years; extensive tophaceous deposits

0.10 atom per cent N^{15} was rapidly reached by the fourth day after ingestion of an equivalent dose of glycine-N^{15}, L. M. and J. B. showed a slower rate of incorporation which did not attain the normal curve until the eighth day and a peak (of approximately 0.13 atom per cent excess N^{15}) on about the fifteenth day. Thereafter the rate curves show a slow decline, reaching the normal level after the fourth week.

In view of the difference in weight of the two patients (L. M., 53.5 Kg.; J. B., 63.7 Kg.) and the consequent disparity in the total dose of glycine-N^{15} administered (L. M., 5.35 gm.; J. B., 6.37 gm.) the rate curves were recalculated in terms of atom per cent excess N^{15} incorporation into urinary uric acid per Kg. body weight. These curves did not differ significantly from Figure 1.

It will be noted in Figure 1 that the rate of glycine-N^{15} incorporation into urinary uric acid in L. M., who had clinically overt gout secondary to polycythemia vera in the myeloid metaplasia phase, did not vary in any essential from that in J. B., who exhibited no clinical manifestations of gout, but both differed strikingly from that in D. R., who had primary gout [*33,34*] with habitual excretion of excessive quantities of uric acid in the urine while on a restricted diet. In Case D. R. the urinary uric acid-N^{15} was almost 0.30 atom per cent excess at the end of twenty-four hours and reached peak abundance, of about 0.35 atom per cent excess N^{15}, by the second day, declining rapidly thereafter. Many cases of primary gout however, probably the majority, show glycine-N^{15} incorporation curves of uric acid biosynthesis in-

TABLE II

DATA ON INCORPORATION OF GLYCINE-N^{15} INTO URIC ACID IN TWO CASES OF POLYCYTHEMIA VERA (L. M., J. B.) AND ONE CASE OF SECONDARY POLYCYTHEMIA (G. D.)

Day of Experiment	Daily Excretion of Uric Acid-N^{15} (Atom % Excess)			Daily Excretion of Uric Acid-N^{15} (μg./24 hr.)			Cumulative Excretion of Uric Acid-N^{15} (mg.)			Cumulative Excretion of Uric Acid-N^{15} (% of ingested N^{15})		
	L. M.	J. B.	G. D.	L. M.	J. B.	G. D.	L. M.	J. B.	G. D.	L. M.	J. B.	G. D.
1	0.0292	0.0210	0.0402	66.0	35.3	39.7				0.011	0.005	0.007
2	0.0499	0.0503	0.0685	87.3	86.0	76.0	0.15	0.12	0.12	0.026	0.017	0.021
3	0.0680	0.0555	0.0809	121.0	102.1	89.8	0.27	0.22	0.21	0.046	0.031	0.037
4	0.0717	0.0601	0.0834	133.4	101.0	88.4	0.41	0.32	0.29	0.068	0.035	0.053
5	0.0721	0.0688	0.0855	129.8	110.8	113.7	0.54	0.44	0.41	0.090	0.061	0.073
6	0.0939	0.0705	0.0855	182.2	114.2	95.8	0.72	0.54	0.50	0.120	0.077	0.091
7	0.0921	0.0802	0.0849	185.1	128.3	84.1	0.90	0.67	0.59	0.151	0.095	0.106
8	0.0980	0.0956	0.0804	170.5	161.6	94.1	1.07	0.83	0.68	0.179	0.118	0.123
9	0.1126	0.1010	0.0838	201.6	167.0	102.2	1.28	1.00	0.78	0.213	0.141	0.141
10	0.1126	0.1039	0.0830	208.3	175.6	91.3	1.49	1.17	0.88	0.248	0.166	0.157
11	0.1235	0.1096	0.0768	212.4	179.7	77.6	1.70	1.35	0.95	0.283	0.191	0.171
12	0.1312	0.1173	0.0775	246.7	218.2	80.6	1.94	1.57	1.03	0.324	0.221	0.186
13	0.1286	0.1145	0.0775	231.5	217.6	83.7	2.18	1.79	1.12	0.363	0.252	0.201
14	0.1276	0.1208	0.0782	229.7	217.4	89.9	2.41	2.00	1.21	0.401	0.284	0.217
15	0.1371	0.1312	0.0787	290.7	232.2	88.9	2.70	2.24	1.30	0.449	0.317	0.233
16	0.1312	0.1271	0.0748	246.7	232.6	88.3	2.94	2.47	1.38	0.490	0.350	0.249
17	0.1165	0.1196	0.0741	189.9	222.5	84.5	3.13	2.69	1.49	0.522	0.381	0.264
18	0.1292	0.1193	0.0741	236.6	214.7	79.3	3.37	2.91	1.55	0.562	0.411	0.281
19	0.1102	0.1141	0.0668	196.2	212.2	61.3	3.57	3.12	1.61	0.594	0.441	0.292
20	0.1126	0.1055	0.0613	182.4	191.0	63.1	3.75	3.31	1.67	0.625	0.467	0.304
21	0.1050	0.1100	0.0578	202.7	198.0	60.7	3.95	3.51	1.73	0.658	0.495	0.315
26	0.0834	.....	.....	126.8	.....	.....	....	....	....	.....	.....	.....
27	.....	0.0830	.....	.....	154.4	.....	....	....	....	.....	.....	.....
28	.....	0.0792	.....	.....	137.0	.....	....	....	....	.....	.....	.....
29	0.0758	.....	.....	171.3	.....	.....	....	....	....	.....	.....	.....
31	.....	.....	0.0398	.....	.....	46.2	....	....	....	.....	.....	.....
33	0.0590	.....	.....	112.7	.....	.....	....	....	....	.....	.....	.....
34	.....	0.0578	.....	.....	99.4	.....	....	....	....	.....	.....	.....
35	.....	0.0594	.....	.....	106.3	.....	....	....	....	.....	.....	.....
36	0.0574	.....	.....	128.6	.....	.....	....	....	....	.....	.....	.....
37	.....	.....	0.0360	.....	.....	33.5	....	....	....	.....	.....	.....
38	.....	.....	0.0298	.....	.....	30.7	....	....	....	.....	.....	.....
39	0.0470	.....	.....	99.2	.....	.....	....	....	....	.....	.....	.....
41	.....	0.0518	.....	.....	83.9	.....	....	....	....	.....	.....	.....
42	0.0427	0.0509	.....	89.7	87.5	.....	....	....	....	.....	.....	.....
49	.....	.....	0.0272	.....	.....	20.1	....	....	....	.....	.....	.....
50	.....	.....	0.0213	.....	.....	25.6	....	....	....	.....	.....	.....
55	.....	0.0395	.....	.....	61.6	.....	....	....	....	.....	.....	.....
56	.....	0.0295	.....	.....	49.0	.....	....	....	....	.....	.....	.....
69	.....	0.0292	.....	.....	53.1	.....	....	....	....	.....	.....	.....
70	.....	0.0162	.....	.....	31.1	.....	....	....	....	.....	.....	.....
72	.....	.....	0.0137	.....	.....	12.5	....	....	....	.....	.....	.....
73	.....	.....	0.0122	.....	.....	12.4	....	....	....	.....	.....	.....
88	.....	0.0170	.....	.....	29.8	.....	....	....	....	.....	.....	....
89	.....	0.0069	.....	.....	13.4	.....	....	....	....	.....	.....	.....
93	.....	.....	0.0064	.....	.....	9.0	....	....	....	.....	.....	.....
94	.....	.....	0.0061	.....	.....	8.2	....	....	....	.....	.....	.....

distinguishable from the normal, with peak abundance on the second to fourth day but not in excess of the normal. Whether or not overproduction of uric acid in such cases occurs from precursors other than glycine, by other metabolic pathways, remains to be established.

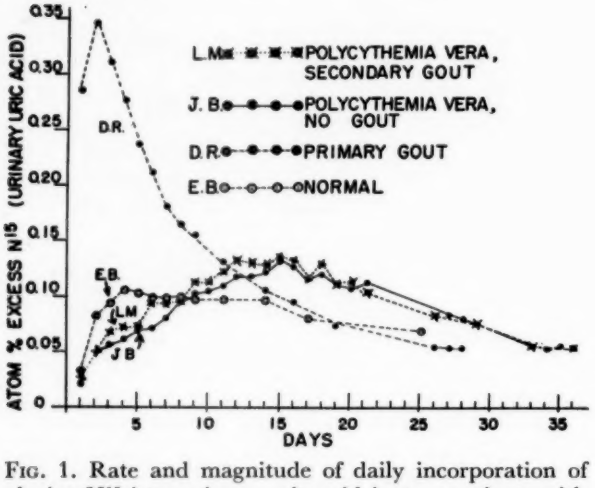

FIG. 1. Rate and magnitude of daily incorporation of glycine-N^{15} into urinary uric acid in two patients with polycythemia vera, one associated with secondary gout (Case L. M.), the other not (Case J. B.). Corresponding curves for a normal human subject (E. B.) and for a patient with primary gout who is an "over-producer" of uric acid (D. R.) are included as standards of reference. All subjects represented received an equivalent dosage of glycine-N^{15}: 100 mg. (60 atom per cent excess N^{15}) per Kg. body weight.

Rate of Incorporation of Glycine-N^{15} into Urinary Uric Acid in Secondary Polycythemia. (Table II, Fig. 2 and Ref. 22.) The rate curves in Cases G. D. and E. F. differ from each other, for reasons not now apparent, and both differ significantly from the rate curves of polycythemia vera. In Case G. D. peak incorporation of glycine-N^{15} into uric acid was reached at about the fifth day and this rate was substantially maintained for about two weeks, followed by a very slow decline. (When recalculated in terms of atom per cent excess N^{15} incorporation into urinary uric acid per Kg. body weight, the curve falls somewhat above that of the normal subject, E. B., but is otherwise not appreciably altered). This subject did not have any clinical manifestations of gout. In Case E. F. the slope of the rate curve of glycine-N^{15} incorporation into uric acid was slightly less than the normal for the first four days but the isotope abundance then became excessive, reaching a peak (of approximately 0.14 atom per cent excess N^{15}) on about the tenth day, declining very slowly after the thirteenth day. This subject had the typical manifestations of (secondary) gout.

Cumulative Rate Curves of Glycine-N^{15} Incorporation into Urinary Uric Acid in Primary and Secondary Gout. (Table II.) Figure 3 depicts the cumulative rate of urinary uric acid-N^{15} excretion, ex-

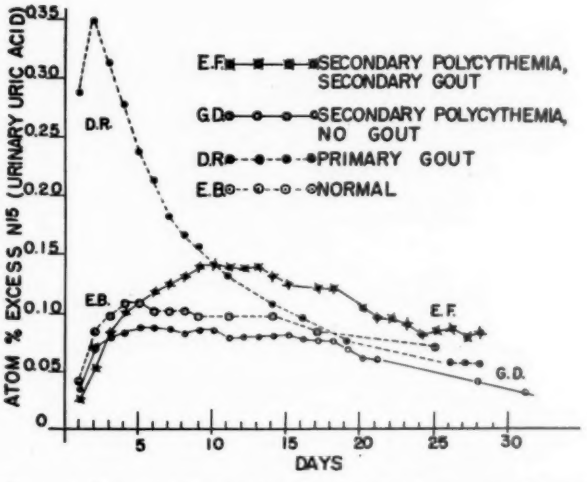

FIG. 2. Rate and magnitude of daily incorporation of glycine-N^{15} into urinary uric acid in two patients with secondary polycythemia, one associated with secondary gout (Case E. F.), the other not (Case G. D.). The same standards of reference are included as in Figure 1. All subjects represented received an equivalent dosage of glycine-N^{15}.

pressed in milligrams (i.e., cumulative urinary excretion of atom per cent excess uric acid-N^{15} *times* cumulative total uric acid nitrogen excreted), in Cases L. M. (polycythemia vera) E. F. (secondary polycythemia), D. R. (primary gout), and in normal subject E. B. These curves indicate more clearly the magnitude and rate of overproduction of uric acid in these several disorders. Excessive biosynthesis of uric acid was most marked in patient D. R. (data given elsewhere [*33*]) who excreted a total of approximately 5.0 mg. uric acid-N^{15} within the first nine days after ingestion of glycine-N^{15} (about five times the normal figure) and 8.5 mg. by the end of four weeks, almost three times the normal quantity. In Case L. M. the total excretion of uric acid-N^{15} in nine days was 1.28 mg. (within the normal range) and increased to 5.1 mg. by the end of four weeks, about 1.5 times the normal. The cumulative curve in Case J. B. (polycythemia vera) paralleled that in Case L. M. but at a somewhat lower level. In Case E. F. the cumulative excretion of uric acid-N^{15} was below the normal for the first ten days, then

somewhat exceeded the normal; at the end of four weeks a total of 3.5 mg. uric acid-N^{15} had been excreted, a quantity slightly greater than the normal. In Case G. D. (secondary polycythemia) the cumulative curve roughly paralleled the normal at a slightly lower level.

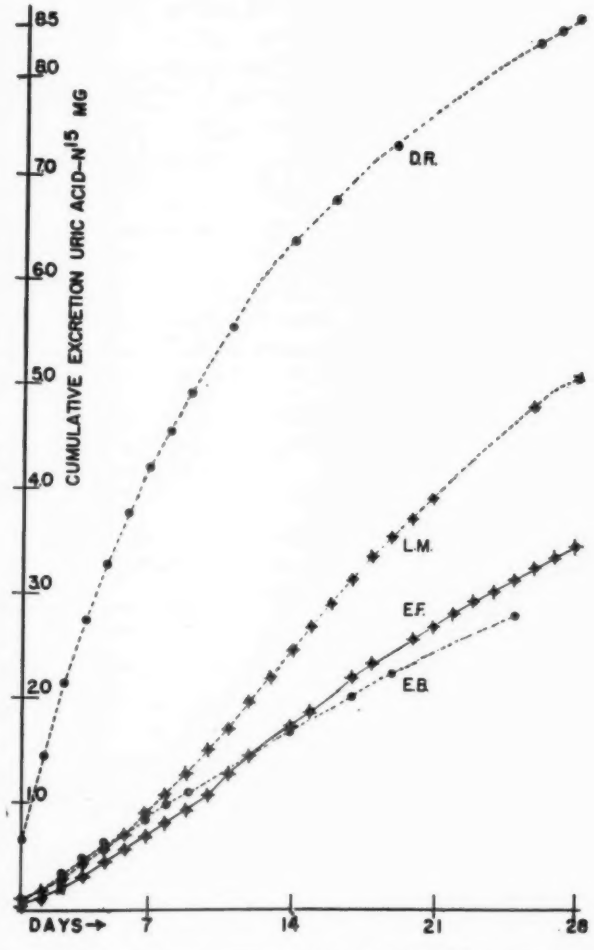

FIG. 3. Cumulative curve of uric acid-N^{15} excretion, expressed in milligrams. Represented are Case L. M. (polycythemia vera, secondary gout), Case E. F. (secondary polycythemia, secondary gout) and, as standards of reference, Case D. R. (primary gout) and normal subject, E. B.

Figure 4 depicts the cumulative rate of urinary uric acid-N^{15} excretion expressed as percentage of glycine-N^{15} ingested. In Case D. R. (primary gout) there was excessive initial diversion of the glycine-N^{15} into the rapid metabolic channels of uric acid biosynthesis; at the end of seven days approximately 0.4 per cent of the ingested N^{15} had been excreted as into uric acid, about four times the normal. After the third week the slope of the curve approximated that of the normal subject E. B. but the cumulative percentage was more than twice the normal. In Case L. M. (polycythemia vera) the percentage of total N^{15} excreted as uric acid-N^{15} was within the normal range for the first week, then sharply increased; at the end of four weeks the cumulative percentage was almost 2.5 times the normal, exceeding that in the patient with primary gout. In the other patient with polycythemia (Case J. B.) a similar but less marked increase was noted. In cases E. F. and G. D. (secondary polycythemia) the curves roughly paralleled the normal but at a slightly higher level.

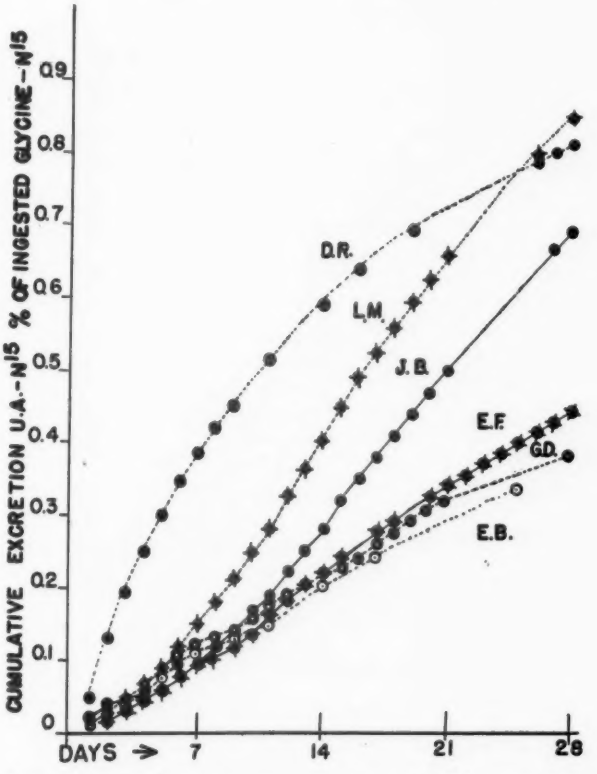

FIG. 4. Cumulative curve of uric acid-N^{15} excretion, expressed as percentage of ingested glycine-N^{15}. Represented are two cases of polycythemia vera, one with associated secondary gout (Case L. M.), the other not (Case J. B.); two cases of secondary polycythemia, one associated with secondary gout (Case E. F.), the other not (Case G. D.); and, as standards of reference, Case D. R. (primary gout) and normal subject, E. B.

Excretion of Purine Bases Other Than Uric Acid in Primary and Secondary Polycythemia. In addition to the studies on uric acid already described, chromatographic methods devised for the separation and semi-quantitation of other purine bases occurring (in small quantities) in the urine of man [*37–41*] were applied. The results are

TABLE III

URINARY EXCRETION OF PURINE BASES (OTHER THAN URIC ACID) IN NINE NORMAL SUBJECTS, FOUR CASES OF POLYCYTHEMIA VERA, TWO CASES OF MYELOFIBROSIS IN THE TERMINAL PHASE AND ONE CASE OF SECONDARY POLYCYTHEMIA*

	Normal		Polycythemia Vera				Myelofibrosis		Secondary Polycythemia
	Mean	Range	L. M.	J. B.	L. L.	Z. S.	E. G.†	F. C.	G. D.
Hypoxanthine	9.7	5.9–13.2	5.6	7.5	2.5	3.4	3.1	1.6	7.5
Xanthine	6.1	5.1– 8.6	3.4	4.7	2.7	3.5	2.6	1.1	3.1
7-Methylguanine } 1-Methylguanine }	6.5	5.5– 7.8	7.3	6.1	2.6	4.8	7.7	3.2	4.2
Adenine	1.4	1.1– 1.7	1.4	1.4	1.5	1.4	1.7	1.7	1.1
Guanine	0.4	0.2– 0.6	0.3	0.7	0.2	0.3	0.3	0.3	0.5
8-hydroxy-7-methylguanine	1.6	1.1– 2.0	3.7	3.3	4.3	3.8	3.7	2.2	1.1
N^2-methylguanine	0.5	0.4– 0.6	...	0.4	...	0.5	0.5	0.5	...
1-Methylhypoxanthine	0.4	0.2– 0.7	0.6	0.4	1.4	1.9	0.9	1.2	0.2
"Spot V"	1.2	0.8– 1.5	1.8	1.4	1.9	2.2	1.9	0.6	0.9
"Spot W"	0.7	0.6– 0.9	1.0	1.4	1.6	1.7	1.7	0.8	...
"Spot S"	0.4	0.2– 0.6	0.6	0.4	0.3	1.4	0.9	0.4	0.3

* Values expressed in mg./day.

† Terminal "subacute myeloblastic leukemia." White blood cell count 47,000 to 66,000 per cu. mm., with 8% myeloblasts, 6% promyelocytes and 15% myelocytes. Red blood cell count 2.0 million per cu. mm., hemoglobin 5.4 gm. %. Sternal bone marrow was hypocellular with increased number of immature myeloid cells.

summarized in Table III. The urinary excretion of 8-hydroxy-7-methylguanine in all four cases of polycythemia vera studied in this connection was found invariably to be increased; the excretion of hypoxanthine and xanthine was almost uniformly decreased; the excretion of 1-methylhypoxanthine, "spot V" and "spot W" (the latter two as yet unidentified) was increased in most instances. Adenine, guanine, 7-methylguanine plus 1-methylguanine (not separated in most instances) and N^2-methylguanine usually appeared in the urine in normal quantities.

In the single patient with secondary polycythemia examined, the urinary excretion of all these components was within the normal range except for some diminution in xanthine and 7-methylguanine plus 1-methylguanine.

TABLE IV

N^{15} CONTENT OF PURINES ISOLATED FROM THE URINE OF J. B., A CASE OF POLYCYTHEMIA VERA, AFTER INGESTION OF GLYCINE-N^{15}

	Urinary Excretion (atom % excess N^{15})	
	Days 1–4 (pooled)	Days 11–14 (pooled)
7-Methylguanine	0.141	0.110
8-Hydroxy-7-methylguanine	0.139	0.118
Adenine	0.118	0.110
Hypoxanthine	0.098	0.122
Xanthine	0.072	0.109
Uric acid	0.047	0.118

Incorporation of Glycine-N^{15} into Purine Bases Other than Uric Acid in Polycythemia Vera. In the glycine-N^{15} feeding experiment in Case J. B. some of these purine bases were isolated from the pooled collection of urine obtained one to four days, and again eleven to fourteen days, after the ingestion of isotope, and the incorporation of N^{15} determined. The results are summarized in Table IV. It will be noted that all the purines so obtained, 7-methylguanine, 8-hydroxy-7-methylguanine, adenine, hypoxanthine and xanthine, were found to contain N^{15}; in fact, in terms of atom per cent excess N^{15} they contained more than uric acid in the sample representing the first four-day collection.

COMMENTS

Glycine is an important precursor or intermediate in the biosynthesis of proteins, nucleic acids, porphyrins, serine, creatinine, glutathione and other vital nitrogenous components, and in the formation of such essential non-nitrogenous metabolites as glycogen and fatty acids. (Fig. 5.) In the overall metabolism of glycine the chief end-product of the oxidation of the carbon atoms is respiratory carbon dioxide, the nitrogen being eliminated for the most part in man as urea, formed ordinarily chiefly by way of serine, and to a relatively small extent as uric acid. The ultimate fate of administered glycine is determined by many factors which affect the proportionate role of the multitudinous alternative pathways which compete for the disposition of glycine. These recently have been reviewed by Arnstein [*50*].

In polycythemia there is some diversion of glycine into the metabolic pathways leading to synthesis of hemoglobin and of nucleic acids in order to meet the increased requirements of excessive hematopoiesis. The absolute quantity of glycine so diverted is small in relation to the total glycine metabolism but may be large in terms of the amount normally utilized for these purposes and, of course, of great significance to the body economy.

Incorporation of Glycine-N^{15} into Hemin in the Polycythemias. The metabolic routes of hemin biosynthesis from glycine and acetate, by way of the succinate-glycine cycle, have recently been elucidated by Shemin [*51,52*] and others [*52*]. Succinyl coenzyme A condenses on the α-carbon atom of glycine to form α-amino-β-ketoadipic acid, then by decarboxylation δ-aminolevulinic acid [*53*], two molecules of which condense to form a monopyrrhole identified as porphobilinogen, from which the tetrapyrrhole is formed by condensation. Glycine is utilized also in the biosynthesis of globin [*54*].

Studies of the incorporation of glycine-N^{15} into hemin in a case of polycythemia vera by London and associates [*55*] revealed a more than two-fold increase in the rate of red cell and hemoglobin production. The mean life span of the red cells in this case was found to approximate the normal, with a normal pattern of red cell destruction. These results are in accord with the conclusion drawn from morphologic studies of the bone marrow [*13,15,19,20*], application of the Ashby technic [*14,56*] and radioactive iron studies [*57,58*]—that the excessive number of erythrocytes in polycythemia vera is not due to prolongation of the life span of the red cell but principally to increased erythropoietic activity. This applies also to secondary polycythemia [*22,59–61*].

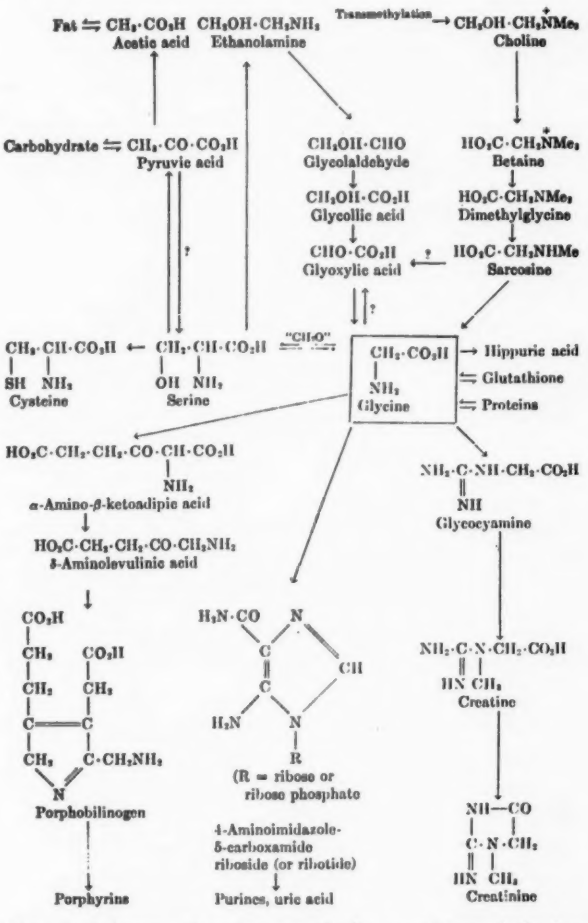

FIG. 5. Schema of major metabolic reactions of glycine, from Arnstein.[50]

Incorporation of Glycine-N^{15} into Uric Acid in Polycythemia Vera. Augmented hemopoiesis in the polycythemias is reflected also in the rate and magnitude of glycine-N^{15} incorporation into uric acid. Some of the intermediary pathways of uric acid biosynthesis have been clarified in recent years, notably the initial synthesis of ribosephosphate derivatives of glycine. From these is formed an intermediate common to divergent routes leading, on the one hand, to formation of "active" adenine and guanine, their incorporation into nucleic acids and ultimate degradative transformation into uric acid; and, on the other hand, to more direct biosynthesis of

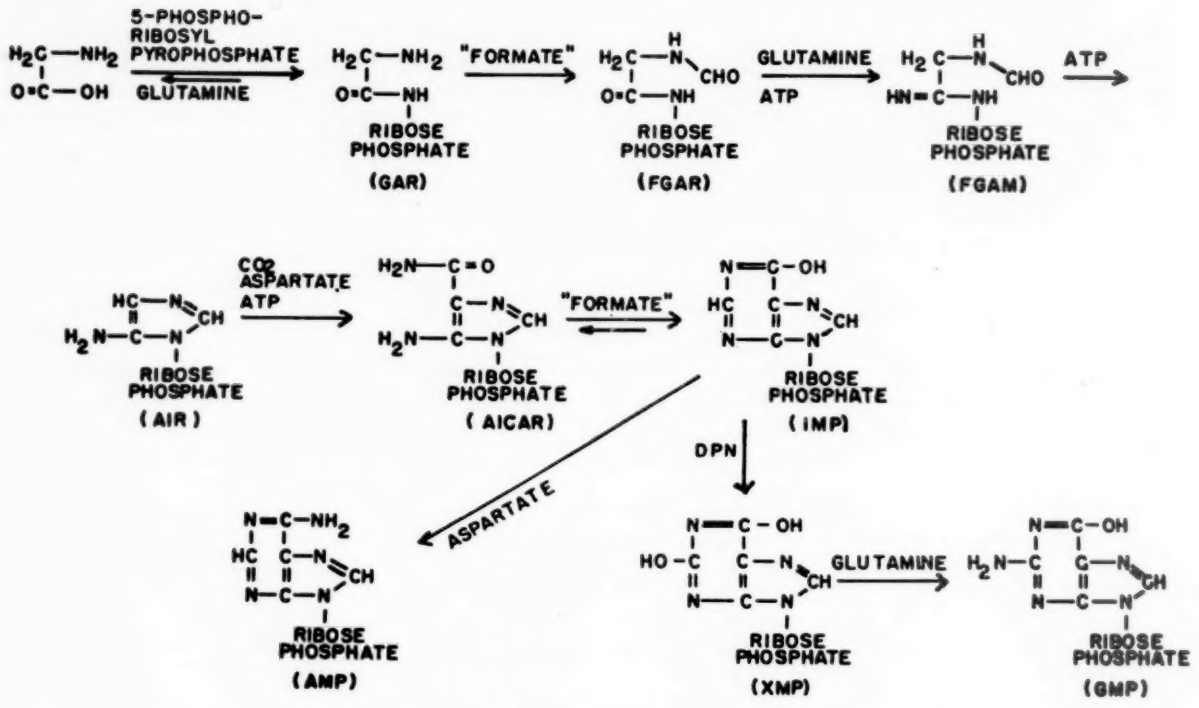

FIG. 6. Schematic representation of steps in the biosynthesis of purines from glycine. Compiled from publications by Buchanan et al. [*62*] (also *J. Am. Chem. Soc.*, 78: 504, 1956); Greenberg et al. [*63*] (also *J. Biol. Chem.*, 219: 423, 1956); Abrams and Bentley, *J. Am. Chem. Soc.*, 77: 4179, 1955; Gehring and Magasanik, *J. Am. Chem. Soc.*, 77: 4685, 1955. GAR = glycinamide ribotide; FGAR = formylglycinamide ribotide; FGAM = formylglycinamidine ribotide; AIR = 5-aminoimidazole ribotide; AICAR = 5-amino-4-imidazolecarboxamide ribotide; IMP = inosinic acid; AMP = adenylic acid; XMP = xanthylic acid; GMP = guanylic acid.

uric acid by routes which by-pass nucleic acid synthesis.

Buchanan [*22*] and Greenberg [*63*], and their associates, using pigeon liver extracts, have demonstrated the initial formation from glycine of glycinamide ribotide, with 5-phosphoribosyl pyrophosphate and glutamine as the respective donors of the ribotidyl moiety and the amide nitrogen. Then follows the successive synthesis of formylglycinamide ribotide, formylglycinamidine ribotide, 5-aminoimidazole ribotide and 5-amino-4-imidazolecarboxamide ribotide. (Fig. 6.)* Closure of the ring of this last compound by formylation in position 2 completes the purine structure to yield inosinic acid. Just where in this sequence is the common intermediate from which the two major pathways of uric acid biosynthesis spring is not yet known but its general position in the biosynthesis of nucleic acids *de novo* from glycine and other small molecule carbon and nitrogen precursors, and its relationship to the incorporation of dietary preformed purines and purine derivatives into nucleic acids [*65*], may be pictured schematically as in Figure 7.

That biosynthesis of uric acid in man occurs by the recently discovered rapid metabolic pathways which by-pass nucleic acid formation, as well as by the long-known biotransformation of exogenous and endogenous nucleic acids, is suggested by the appearance of N^{15}-labelled uric acid in the urine within a few hours after administration of glycine-N^{15}. Indeed, normally these "shunt" pathways may well quantitatively predominate since the urinary uric acid reaches a peak isotope abundance—approximately 0.10 atom per cent excess N^{15} after feeding 100 mg. glycine-N^{15} (60 atom per cent excess N^{15}) per Kg. body weight—within two to four days, after which the uric acid N^{15} excretion very slowly declines over a period of many weeks.

A consistently different pattern was obtained in the two patients with polycythemia vera (L. M., J. B.) herein described. The specific

* Evidence for the intermediary position of 5-amino-4-imidazolecarboxamide (presumably as the ribotide) in uric acid biosynthesis in man has been procured by Stetten and his associates [*64*].

activity of the urinary uric acid was less than the normal in the first few days after ingestion of an equivalent dosage of glycine-N^{15}; progressive enrichment of urinary uric acid-N^{15} occurred more slowly, peak abundance (0.13 atom per cent excess N^{15}) not being reached until about the fifteenth day; then there was a very slow decline over a period of weeks, as in the normal. The low initial concentration of excess N^{15} in the urinary uric acid, which was noted also by Laster and Muller [*36*] in their case of agnogenic myeloid metaplasia, probably is not accounted for solely by dilution with newly formed, unlabeled uric acid since the total uric acid nitrogen excretion was not excessive. It may therefore be concluded that there was some diversion of glycine from the rapid "shunt" pathways of uric acid biosynthesis to the slower metabolic routes involving incorporation into intracellular nucleic acids, a redistribution consistent with the exaggerated hemopoietic requirements of this disorder. Moreover, the abnormally high percentage of ingested glycine-N^{15} appearing in the urinary uric acid implies some over-all diversion of glycine from pathways not leading to uric acid but, presumably, to urea formation.

The data on the cumulative incorporation of glycine-N^{15} into urinary uric acid in these two patients with polycythemia vera unequivocally demonstrate overproduction of uric acid. This doubtless accounts for the hyperuricemia consistently observed in these patients (Case L. M., 12.1–14.4 mg. per cent; Case J. B., 8.2–8.7 mg. per cent) and the urinary excretion of uric acid near the upper limits of normal (565 and 560 mg. per twenty-four hours, respectively, on a low purine, restricted protein diet). No data are available on the rate and magnitude of glycine-N^{15} incorporation into uric acid in polycythemia vera not accompanied by hyperuricemia; such cases may give less, perhaps no indication of overproduction of uric acid.

The steps by which uric acid becomes the major end-product, in man, of the catabolism of the purine components (adenine and guanine) of intracellular nucleic acids have not been fully elucidated [*66,67*]. Nevertheless it may be presumed that the chief rate-determinant in uric acid formation from this source is the fate of the cell containing the nucleic acids; in polycythemia, the time required for elaboration and extrusion of the nuclear elements of red cell precursors, in the case of polycythemia vera and myeloid metaplasia perhaps also of myeloid and megakaryocytic elements. So considered, the time curves of glycine-N^{15} incorporation into urinary uric acid in polycythemia may provide a rough indication of the life span of erythrocyte and other blood cell precursors, much as the time curves of glycine-N^{15} incorporation into circulating hemin provide a measure of the life span of the circulating erythrocyte.

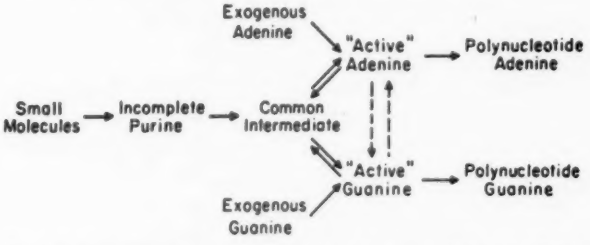

FIG. 7. Schematic representation of the position of the "common intermediate" in the synthesis of polynucleotide purines, from G. B. Brown, *Federation Proc.*, 15: 823, 1956.

Incorporation of Glycine-N^{15} into Uric Acid in Secondary Polycythemia. The glycine-N^{15} incorporation curves of the two cases of secondary polycythemia studied (Cases E. F., G. D.) differ from those obtained in polycythemia vera. It is possible but improbable that variations in the rate of erythrogenesis alone account for these differences, particularly since the rate curves for the two patients with polycythemia vera (Cases L. M. and J. B.) and for Laster and Muller's patient with agnogenic myeloid metaplasia [*36*], whose several hemograms suggest that they did not have identical rates of erythrogenesis, were virtually superimposable. The differences between primary and secondary polycythemia appear rather to include differences in metabolic pathways of hematopoiesis, and may be related to the enhanced production of cells of the myeloid series characteristic of the late phases of the myeloproliferative disorders. Our two cases of polycythemia vera and Laster and Muller's case of agnogenic myeloid metaplasia all gave evidence of increased myeloid activity in peripheral blood smears and direct bone marrow examination. This thesis is further supported by the results of studies of the urinary excretion of purines other than uric acid.

The glycine-N^{15} incorporation curves in the two cases of secondary polycythemia also differed from each other. Clarification of the significance of these variations must await further study.

Relation of the Rate and Magnitude of Glycine-N^{15} Incorporation into Uric Acid to the Presence or Absence of Clinical Manifestations of Secondary Gout. It is becoming increasingly apparent that gout, like some other metabolic diseases, is a disorder of multiple pathogenesis. Although the clinical manifestations and therapeutic responses of acute gouty arthritis and chronic tophaceous deposits in primary and secondary gout are indistinguishable, the predominant mechanisms of biosynthesis of uric acid clearly are different [*22,35,68*]. Primary gout, the classic form of the disease, is an inborn error of metabolism; when excessive production of uric acid has been demonstrated by glycine-N^{15} feeding [*33,34*], this has occurred predominantly by direct metabolic pathways not involving augmented nucleic acid formation and breakdown. (However, as already indicated, excessive biosynthesis of uric acid from glycine-N^{15} is not demonstrable in some, probably the majority of patients, with manifest primary gout [*34*]). Secondary gout, on the other hand, is defined as an acquired complication particularly of certain hemopoietic disorders in which, incidental to markedly accelerated turnover of nucleic acids related to overproduction of one or another type of (blood) cell, there is excessive biosynthesis of uric acid and other purines by slow, indirect metabolic pathways involving increased formation and degradation of nucleic acids.

The results of the present study support this concept of the origin of secondary gout. It should be noted, however, that while the rate and magnitude of glycine-N^{15} incorporation into uric acid were very similar in Cases L. M., J. B. and Laster and Muller's case of agnogenic myeloid metaplasia, J. B., unlike the other two patients, never exhibited any clinical manifestations of gout, despite persistent hyperuricemia—it would be impossible, from the rate and cumulative curves of glycine-N^{15} incorporation into uric acid, to prognosticate whether clinically overt gout was or was not present. This applies also, perhaps less convincingly, to the findings in secondary polycythemia. And, as already indicated, the same situation obtains in primary gout.

These discrepancies between the magnitude of uric acid biosynthesis and the presence or absence of clinically overt gout point up certain ambiguities in the definition of gout, particularly of secondary gout. The presence of hyperuricemia and/or hyperuricosuria in association with hemopoietic and related disorders does not of itself justify the diagnosis of secondary gout. In order to avoid even greater confusion this should be reserved, for the present, for the relatively few patients who give a history of acute gouty arthritis or, perhaps, exhibit tophaceous deposits. Such a restriction of diagnostic criteria would not apply in primary gout and admittedly is not altogether satisfactory in secondary gout.

The discrepancies noted also call attention to certain misconceptions concerning the proximate causes of the clinical manifestations of gout, particularly of acute gouty arthritis. As set forth elsewhere [*69,70*], there is no convincing evidence that uric acid *per se*, a pharmacologically inert substance, is the immediate cause of the acute gouty seizure, despite the traditional views on this point, and much to indicate that it is not; one should not be surprised, therefore, to encounter excessive uric acid biosynthesis without acute gouty arthritis, for example in the intercritical phase of primary gout. In respect to the relationship between the rate of uric acid biosynthesis and the rate of formation of tophi, such a relationship exists, of course, but the adequacy of renal excretion of uric acid often appears to be the decisive rate determinant in tophus formation.

A clinically important complication of excessive uric acid production in hemopoietic and other disorders involving augmented turnover of nucleic acids is the precipitation of uric acid in the kidneys and urinary outflow tract, with obstruction. This hazard is increased by acceleration of nucleic acid degradation by radiation therapy and administration of chemotherapeutic agents [*71–73*]. Case L. M. illustrates the marked rise in serum and urinary uric acid concentrations which may ensue.

Urinary Excretion of Purines Other than Uric Acid in the Polycythemias. The significance of the variations from the normal observed in the urinary excretion of purines other than uric acid (Table III), and indeed of the normal occurrence of these compounds in the urine, is not known. Guanine, 1-methylguanine, 8-hydroxy-7-methylguanine, N^2-methylguanine and 1-methylhypoxanthine were but recently isolated for the first time from urine, the last four for the first time from any biologic source [*37–41*]. The new purines appear to be endogenous metabolites but their metabolic significance is, as yet, only a matter of surmise [*41*]. Of special

interest in this connection is 8-hydroxy-7-methylguanine, which was unequivocally demonstrated to be an endogenous metabolite by glycine-N^{15} incorporation. Excessive quantities of this purine were found in the urine in polycythemia vera and in the terminal "leukemic" phase of myelofibrosis, but not in a case of secondary polycythemia. The urinary excretion of 8-hydroxy-7-methylguanine is increased also in the acute gouty attack, but apparently not in the intercritical phase of primary gout [*68*].

SUMMARY

1. The rate and magnitude of glycine-N^{15} incorporation into urinary uric acid was studied in two cases of polycythemia vera and two cases of polycythemia secondary to congenital heart anomaly.

2. It was demonstrated, by the magnitude of cumulative isotope labeling of urinary uric acid, that the occurrence of hyperuricemia and/or hyperuricosuria in some cases of polycythemia is attributable to overproduction of uric acid, as has long been suspected.

3. The rate curves of glycine-N^{15} incorporation into uric acid in polycythemia vera revealed slow, progressive enrichment of isotope, with peak abundance at the end of the second week after glycine-N^{15} ingestion. The predominant route of uric acid biosynthesis in polycythemia vera therefore does not involve rapid, "shunt" metabolic pathways. The time relationships, however, are consistent with conversion of glycine into the purine components of intracellular nucleic acids, and subsequent biotransformation into uric acid. Evidence was obtained of diversion of excessive quantities of glycine into these metabolic channels, presumably to meet the excessive requirements of augmented hemopoietic activity.

4. The rate curves of glycine-N^{15} incorporation into urinary uric acid in secondary polycythemia were found to differ significantly from those in polycythemia vera, at least in the late phases examined. These differences may be related to the excessive production of cells of the myeloid series associated with the late phases of the myeloproliferative disorders.

5. The sharp contrast between the rapid peak of glycine-N^{15} incorporation into uric acid in primary gout and the slow peak in secondary gout indicates that, despite the similarities in clinical manifestations and therapeutic responses, the predominant pathways of uric acid biosynthesis in primary and secondary gout are different, and hence they have a different pathogenesis.

6. It is not possible, from the rate and cumulative curves of glycine-N^{15} incorporation into uric acid, to determine whether or not clinically overt gout has developed or will develop. This apparent paradox, which applies to latent and manifest primary and secondary gout, is discussed.

7. The urinary excretion of purine bases other than uric acid was studied in primary and secondary polycythemia. Excessive excretion of 8-hydroxy-7-methylguanine, a newly discovered purine demonstrated by glycine-N^{15} incorporation to be an endogenous metabolite, was found in polycythemia vera.

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Review

Acute Manson's Schistosomiasis*

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A series of infections among American soldiers in the Far Eastern Theater of World War II led to increasing interest in the problem of acute schistosomiasis. Clinical observations emphasized the very high morbidity of acute infection with Schistosoma japonicum exemplified by the Leyte experience with military personnel.[1,2]

The acute phase of Manson's schistosomiasis has been studied, among others, by Pons in Puerto Rico (twelve cases);[3,4] Girges in Egypt (nine cases);[5] Pifano and Mayer in Venezuela (three cases);[6] Ritchken, and Ritchken and Gelfand in Central Africa (two cases);[7,8] and by Lawton[9] among Australian troops in Egypt. Epidemics of acute Manson's schistosomiasis among previously unexposed men, comparable to those of S. japonicum, have not been observed.

The pathogenesis of acute Manson's schistosomiasis is difficult to analyze because of the scarcity of clinically detectable cases and the lack of pathologic material from human cases. Evidence from experimental studies in the rat,[10,11] rabbit,[10–12] hamster[10,12] and monkey[13] would indicate that the pathologic alterations are dominated by vascular damage, obstructive vascular phenomena and necrosis from the local effects of the metacercariae, the adult parasites, and their eggs. Observations on the more chronic pathologic changes in man tend to support the experimental findings.[14,15]

The accepted views on the life cycle of the parasite in the human host will be summarized. The cercariae penetrate through the skin or mucous membranes. In some instances their entrance may be accompanied by mild pruritus and a punctate erythema. The metacercariae (the cercariae minus their tail) migrate through the lymphatic and venous streams to the lungs whence they pass to the left side of the heart, the general circulation and, finally, reach the intrahepatic portal veins where each one matures into a male or female adult worm. Maturity is reached within twenty-seven or twenty-eight days after entry and, within a variable period ranging from twenty-one to forty-two days, the acute symptomatology appears with fever, allergic manifestations with eosinophilia, cough, abdominal pain, hepatomegaly and splenomegaly.[3–9]

The time required for development of the characteristic local lesions is variable depending on the severity of the reaction of the tissues to irritation caused by eggs and/or parasites, the intensity of the infection and the condition of the host. In some instances the pathologic alterations appear to be cumulative, not infrequently leading to progressive tissue changes.

There is considerable experimental and clinical evidence in support of invariable involvement of the liver in Manson's schistosomiasis. Following exposure the invading cercariae are eventually carried by the blood stream to the intrahepatic radicles of the portal system where full maturation occurs. The adult worms copulate in the mesenteric, colonic and hemorrhoidal vessels, and, after fertilization, the female worm further migrates to smaller branches of those veins, mostly in the submucosa or adjacent portions of the mucosa of the distal colon and rectum, for oviposition. Some of the eggs are swept with the venous circulation of the portal system to the liver. Extensive morphologic and physiologic hepatic alterations may supervene, as a result of either direct or indirect effects of

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the adult parasites and their ova.[5] These have been repeatedly demonstrated in chronic schistosomiasis mansoni in man.[14,15]

The lack of information concerning the earliest hepatic alterations in man results from the relative benignity of the acute phase and the infrequency with which cases are seen so early in the disease. The paucity of reports in the world literature illustrates the rather infrequent recognition of the disease at this stage. The lack of pathologic data on the early "toxemic" stage of the disease is explained by its non-fatal nature at that stage. The information obtained from the experimental animal has been limited to gross and histopathologic studies, and do not include determinations of hepatic function.

Evaluation of the effectiveness of therapy is rendered difficult by the protean clinical manifestations and invariable tendency to chronicity of Manson's schistosomiasis. A review of the published data fails to reveal any drug of invariable efficacy; none is known effectively to control the ravages of the disease. After maturation and oviposition of the parasite have occurred, progressive and at times irreversible tissue alterations may occur, emphasizing the importance of early recognition of the disease in determining the degree of success in management. The direct contact of eggs and/or adult parasites with the host's tissues leads to pathologic alterations which may remain unaltered or only abbreviated by treatment in the earliest phases of the disease. Organization is at times the only possible effective body defense. The prevention of additional pathologic alterations through efficient control of oviposition is attained only by destruction of the adult female parasite which is consequently the main objective of early therapy. The experience of others, like our own, emphasizes the limitations of most therapeutic agents in common use.

The discovery of the causative agent in 1851,[16] led to increased attempts at cure but these were unsuccessful. It was not until 1913,[17] that favorable results with emetine hydrochloride began to appear in the literature. Early enthusiasm for this drug[18] was soon lessened by recognition of the dangerous side effects of the drug, including toxic myocarditis and peripheral neuritis.

The introduction of potassium antimony tartrate (tartar emetic) in 1917,[17] as an effective agent in the treatment of both schistosomiasis mansoni and haemotobium led to further trials with other antimony compounds and to the subsequent development of most of the antischistosomal agents used at present. It must be emphasized that tartar emetic is regarded as the most potent antischistosomal agent.[19] However, the drawbacks of its toxic reactions (nausea, vomiting, dizziness, epigastric pain, diarrhea, arthralgia and coughing), method of administration (intravenously, at times leading to phlebitis), and its low chemical stability when in solution have limited its use except under the most favorable conditions. Furthermore, sudden death from the intravenous injection of tartar emetic is not a rarity (0.5 per cent in a series of 1,000 cases).[20,21]

By 1929, reports[22] started to appear in the literature on the use of antimony pyrocatechol sodium disulphonate (fuadin®) (stibophen) in the management of S. mansoni and S. haematobium infections. Several attempts to evaluate the effectiveness of the drug in the chronic phase of schistosomiasis have appeared in the literature.[23–27] There is little information on the effect of the drug in the earliest phases of the infection and, indeed, there is altogether a limited number of studies on the acute phase of the disease.[3,4,6]

This drug is recognized to be less toxic and more chemically stable than tartar emetic, and it can be administered intramuscularly with little if any local irritation. Experience has demonstrated that the doses recommended by the early workers (40 to 50 cc. in nine to ten intramuscular injections) are usually inadequate in the management of the disease.[28]

We consider it timely to report on twelve cases of acute Manson's schistosomiasis studied in Puerto Rico during the past six years. An attempt is made to correlate the pathologic, immunologic and clinical manifestations from the outset of the disease and to evaluate the effects of early fuadin therapy by close follow-up of treated patients.

RESULTS

Clinical Picture. All patients were males, ten white and two mulatto; the youngest was eight, the oldest sixteen, with an average age of 12.8 years. Infection followed exposure in heavily contaminated streams during the spring, summer and early autumn in bright daylight of mid-morning or afternoon. The incubation period (time elapsing from first exposure to clinical onset) varied from twenty-one to forty-

two days and averaged 28.3 days. The duration of illness prior to hospitalization averaged fifteen days, extending from four to thirty-four days. Thus the clinical study was initiated within the first thirty-two to sixty-two days after exposure. (Table 1.)

TABLE 1
CORRELATION OF NUMBER OF EXPOSURES WITH CUTANEOUS MANIFESTATIONS, INCUBATION PERIOD AND DURATION OF FEVER

Case	Exposures	Early Cutaneous Manifestations	Incubation (days)	Duration of Fever (days)
1	1	No	28	63
2	1 (evanescent)	Yes	28	24
3	3	No	30	30
4	1	No	28	21
5	16 (consecutive)	No	28	74
6	25 (consecutive)	No	26	29
7	24 (consecutive)	No	27	23
8	24 (consecutive)	No	26	38
9	24 (consecutive)	No	26	38
10	several (consecutive)	Yes	42	17
11	1	Yes	30	26
12	1	Yes	21	33
			Average 28	Average 35

The criteria for diagnosis were: (1) a history of first exposures to contaminated waters; (2) symptoms and signs consistent with the clinical picture of the disease; (3) laboratory confirmation, including the demonstration of living eggs of S. mansoni in stools and/or rectal mucosa; and (4) demonstration by biopsy specimen of the earliest hepatic lesions with the characteristic eggs. Other infectious or febrile illnesses with a similar clinical picture were excluded by adequate laboratory investigation.

The disease was mistaken for typhoid fever in six instances; in others, including Cases 6, 7, 8 and 9 (the patients were brothers with a reliable history of simultaneous exposure to the same stream, which was known to be contaminated) acute schistosomiasis was suspected from the onset. In two, the clinical impression of bronchopneumonia was entertained until the discovery of marked eosinophilia, which was the main factor pointing to a parasitic infection in every instance.

Exposure to infested streams varied from a few minutes to several hours daily for as long as twenty-five consecutive days. Immediate cutaneous manifestations were mild itching and urticaria, which appeared in four instances and lasted for ten minutes to two hours. Coughing, conspicuously absent during the incubation period, was delayed until the clinical onset of the disease.

The onset was characterized by its explosiveness and dominated by chills, fever, profuse diaphoresis, generalized body aches and pains, headache, anorexia, severe watery diarrhea, non-productive cough and rapid weight loss. Delayed signs of allergy were recorded in six instances: transitory puffiness of eyelids and face in four, urticaria in one and purpura in another.

All the patients were acutely and severely ill when first seen; six showed moderate malnutrition. Spiking, remittent fever was invariably present, with daily afternoon elevations to 105°F. and lower or normal temperatures in the evening. Defervescence was accompanied by marked diaphoresis and extreme prostration. The initial febrile period (six to forty-three days) was followed by remissions of one to eight days, alternating with usually lower and irregular spikes lasting from one to seven days. Fever subsided in seventeen to seventy-four days (average thirty-eight days) in eight untreated patients. Treatment failed to abbreviate the acute phase, with persistence of fever for twenty-nine to forty-two days (average thirty-nine days). The fever invariably subsided by lysis, either spontaneously or after therapy, and its duration after fuadin (stibophen) averaged eighteen days (ten to twenty-five days).

Nausea and vomiting, present since onset in nine patients, lasted for seven to fourteen days. Diarrhea was invariably present. The stools were watery, brownish and mucoid and in three patients they were bloody. The diarrhea was always accompanied by diffuse abdominal pain, and by tenesmus in nine cases, and could be easily mistaken for that of bacillary dysentery. It lasted from one to seven days in four patients; for ten to fifteen in five, and persisted for twenty-two, twenty-three and eighty-five days in each

of three patients. In Case 1, watery diarrhea alternated with soft stools during variable periods covering days and weeks. In Case 4 it subsided on the thirty-ninth day after exposure, to reappear several months after inadequate therapy with fuadin (stibophen).

TABLE II
SYMPTOMS AND SIGNS

Chills	12	Anorexia	10
Fever	12	Tenesmus	9
Diarrhea	12	Nausea	9
Abdominal pain	12	Vomiting	9
Cough	12	Headache	7
Body aches	12	Bronchospasm	4
Weakness	12	Puffiness of eyelids	4
Diaphoresis	12	Early cutaneous signs	4
Prostration	12	Bloody diarrhea	3
Lymphadenopathy	12	Mental dullness	3
Hepatomegaly	12	Arthralgia	2
Weight loss	11	Delayed skin rash	2
Splenomegaly	10	Purpura	1

While none of the patients presented pulmonary manifestations during the incubation period, all suffered from mild to moderately severe, non-productive cough at onset. Two showed signs of bronchopneumonia at the onset and, although the pulmonary findings subsided within several weeks, persistent and intercurrent bouts of bronchospasm were recorded in four instances.

There were no signs of either meningeal or cerebral inflammation, although six patients suffered from moderately severe frontal headache during the first two weeks of illness, and three from mental dullness, rendering the clinical impression of typhoid fever more tenable. Rapid weight loss was recorded in eleven patients and profound anorexia in ten. Weakness, lassitude and generalized body aches and pains were invariably present, while two patients suffered from arthralgia without evident joint alterations.

An impressive finding was that of generalized enlargement of lymph nodes as an invariable occurrence. The nodes were slightly to moderately enlarged, discrete, not tender, and of rubbery consistence. In ten of the patients the spleen was palpable, soft and not tender. The liver was enlarged, soft and tender in every instance but there was no clinical or laboratory evidence of jaundice. (Table II.)

The leukocyte count on admission varied from 8,500 to 18,600, with 20 to 70 per cent eosinophils. Particularly high eosinophilic levels were observed following the first intramuscular injections of fuadin (stibophen), concomitant with a further but transitory hepatic enlargement in seven instances. Although the persistence of the eosinophilia was variable (a few weeks to over a year), it was generally unaltered by therapy.

The initial hemoglobin values were under 10 gm. in one and over 13 gm. in two instances. Decrease in red cells and hemoglobin, with lowest values on the eighth and fifteenth weeks after infection, was invariably observed, and in six patients a frank normochromic anemia developed. An accelerated erythrocyte sedimentation rate was persistently and invariably present. Bone marrow studies showed only an increase of eosinophils.

On sigmoidoscopic examination the rectal and lower intestinal mucosa appeared diffusely edematous and red, with pin-point, yellowish elevations, punctate hemorrhages and small, shallow and clean ulcerations. These early manifestations closely simulated the enanthem of scarlet fever. The alterations gradually subsided during the eighth and tenth weeks after infection, leaving a shiny, finely granular mucosa.

While eggs of S. mansoni were found in the stools as early as the thirty-eighth and usually by the fortieth day, the results from rectal biopsy specimens were equivocal. Thus of the serial rectal biopsy specimens in eleven patients, five were positive initially, while in six positive results were not obtained for one to six weeks. This would suggest that in the early phase of Manson's schistosomiasis a stool examination is the most dependable diagnostic measure.

CASE REPORTS

CASE 6: G. A., a twelve-year old, white Puerto Rican boy, bathed for twenty-five consecutive days in an infested stream. Twenty-six days after the initial exposure chills, fever, headache, transitory urticarial rash, general body aches and pains, crampy abdominal pains, watery bloody diarrhea with tenesmus, dry cough and anorexia developed and there was a weight loss of 12 pounds in fourteen days. He was admitted to the hospital on the thirty-ninth day after exposure, the thirteenth day of illness.

On physical examination he appeared severely undernourished and acutely ill, with a temperature of 100.6°F., a pulse of 80, respirations of 20 per minute, and a blood pressure of 80 mm. Hg systolic and 60 diastolic. Physical examination disclosed moderate, generalized lymphadenopathy, wheezes over both lung fields, a soft liver and a spleen felt 2 cm. below the costal margins.

The laboratory findings between the sixth and twenty-first week following infestation showed hemoglobin values of 9 to 11.2 gm. per cent; leukocyte counts of 6,600 to 28,000 per cu. mm., of which 2,000 to 20,000 were eosinophils; abnormally high serum globulin and quantitatively normal albumin; positive cephalin cholesterol flocculation test until the seventeenth week; slightly abnormal thymol turbidity and bromsulphalein tests; normal prothrombin time and accelerated erythrocyte sedimentation rate. Schistosome eggs appeared in the stools on the fortieth day and in the rectal mucosa on the forty-first. Stool and blood cultures, Widal, Weil-Felix and brucella agglutinations were negative. A false positive Wassermann test appeared on the fortieth day after exposure and reverted to normal during the twelfth week after exposure. The Kahn test was repeatedly negative. No malarial parasites or amebas were demonstrated. X-rays of the chest showed a healed primary tuberculous complex. Rectosigmoidoscopic studies on the forty-first day revealed an edematous, granular mucosa with numerous pin-point hemorrhages which persisted until the sixty-third day. A cercarial antigen skin test was positive on the forty-first day. The adult worm antigen skin test was negative until the forty-eighth day, at which time it became positive.

The diarrhea, cough and wheezes, and headache subsided shortly after admission. The intermittent, spiking fever abated by the fifty-fifth day after infestation and twenty-ninth day of illness. A course of fuadin (stibophen) of 99.0 cc. was initiated on the forty-fifth day, in intramuscular doses of 1.0, 2.0 and 3.0 cc. on alternate days for six doses, and 5 cc. every other day for fifteen doses. Following the first injections the liver increased in size to 4 cm. below the costal margin, with a concomitant increase in the circulating eosinophils. The schistosome eggs disappeared from the stools on the fourteenth day after initiation of therapy. Clinical improvement soon followed with increased appetite and weight gain. The liver and spleen were within normal in size by the twenty-first week after infestation and fifteenth week after therapy.

Seven months after infestation the patient was clinically well and the stools were free of eggs. Fifteen months after infestation he continued well. The hemoglobin was 13 gm. per cent, white blood cell count was 8,050 per cu. mm. and eosinophils, 1,931. Skin tests with cercariae, adult worm and egg antigens were positive. Stool examination failed to reveal eggs. A two-year follow-up demonstrates the persistence of live S. mansoni eggs in the stools and rectal mucosa.

Case 7: A. A., a ten-year old white Puerto Rican boy, bathed in a stream heavily contaminated with S. mansoni for twenty-four consecutive days. Twenty-seven days after the initial exposure chills, fever, headache, general body aches and pains, dry cough, crampy abdominal pain, watery bloody diarrhea with tenesmus, weakness, anorexia suddenly developed and there was a loss of 9 pounds in twelve days. The persistence of symptoms required his hospitalization on the thirty-ninth day after the original exposure, when he showed a temperature of 102°F., pulse of 98, respirations of 22 per minute, and a blood pressure of 110 mm. Hg systolic and 70 diastolic. He was markedly undernourished and acutely ill. There was a definite, generalized, non-tender enlargement of lymph nodes. The liver was soft, not tender, and extended for 2 cm. below the right costal margin. The spleen was soft, not tender, and could be felt 1 cm. below the left costal border.

Laboratory studies between the sixth and twenty-first weeks revealed 8.7 to 11.2 gm. per cent of hemoglobin; 9,100 to 18,300 leukocytes per cu. mm., of which 2,300 to 8,900 were eosinophils; significant increases in serum globulin with normal albumin; positive cephalin cholesterol flocculation tests until the seventeenth week; positive thymol turbidity test; slight retention of bromsulphalein in forty-five minutes, and an accelerated erythrocyte sedimentation rate. Schistosome eggs appeared in the stools on the thirty-ninth day after exposure (first stool examination) and in the rectal mucosa on the seventy-seventh day. Stool and blood cultures were negative for bacterial pathogens. Widal, Weil-Felix and brucella agglutinations, blood Kahn tests and the prothrombin time were negative. No malarial parasites or amoebas were found on repeated tests. A cercarial antigen skin test was positive on the forty-second day. The adult worm antigen skin test became positive on the eighty-fourth day.

Rectosigmoidoscopic studies on the forty-first day revealed generalized edema of the mucosa and numerous pin-point hemorrhages. The hemorrhagic manifestations disappeared spontaneously by the forty-fourth day, with persistence of the edema until the sixty-third day. It must be emphasized that it was not until the seventy-seventh day that the rectal biopsy specimen revealed schistosome eggs.

The diarrhea and cough subsided spontaneously within a few days after admission. The spiking fever persisted until the fiftieth day after exposure and twenty-third day after onset, with persistent low-grade increases until the sixty-ninth day. A course of fuadin (stibophen) totalling 99 cc., in intramuscular doses of 1.0, 2.0 and 3.0 cc. daily, followed by 3.0 cc. on alternate days for six doses, and then increased to 5.0 cc. every other day, was started on the forty-fourth day. A definite increase in liver size, accompanied by an elevation in the eosinophil count, was observed five days after the first injection. Definite clinical improvement rapidly followed, with disappearance of eggs from the stools within thirty-three days, and reversion to normal of the spleen and liver by the twenty-first week after exposure.

Seven months after infestation, repeated stool examination and rectal biopsy specimens failed to reveal eggs. The liver was palpable 1 cm. below the

costal border, but the spleen was not. Fifteen months after infestation the liver was barely palpable, and the stools were negative for live eggs. The hemoglobin was 11.6 gm. per cent, and the white blood cell count 9,100 per cu. mm., with 1,250 eosinophils per cu. mm. of blood. Cercariae, adult worm and egg antigen skin tests were positive. Two years after infestation he was clinically well, but showed live S. mansoni eggs in the stools and rectal biopsy specimens.

Case 8: N. A., a nine-year old white Puerto Rican boy, was exposed to contaminated water for twenty-four consecutive days. Twenty-six days after the initial exposure chills, fever, profuse perspiration, dry cough, severe general malaise with generalized body aches and pains, nausea, vomiting, crampy abdominal pains, watery diarrhea with tenesmus and anorexia developed and there was weight loss. On his admission to the San Juan City Hospital on the thirty-eighth day after the initial exposure and twelfth day of illness he appeared acutely ill, with a temperature of 102°F., pulse of 100 and respirations of 26 per minute. He showed a generalized lymphadenopathy, a soft non-tender liver extending 2 cm. below the right costal margin, and a barely palpable, non-tender spleen.

Studies during the sixth to the twenty-first week after exposure showed hemoglobin values of 8.7 to 10.2 gm. per cent; leukocyte counts of 6,800 to 18,800 per cu. mm. with eosinophil counts of 900 to 12,400; hyperglobulinemia with hypoalbuminemia; significantly positive cephalin cholesterol flocculation test until the eleventh week; 12 per cent bromsulphalein retention in forty-five minutes in the sixth week, and an accelerated sedimentation rate. Schistosome eggs appeared in the feces on the forty-first day (first examination), and in the rectal mucosa on the sixty-sixth day. Urinalyses, prothrombin times and blood and stool cultures were repeatedly negative. Widal, Weil-Felix and brucella agglutinations were negative, and neither malarial parasites nor amebas were found. The blood Kahn test was negative. Radiographs of the chest revealed a healed primary tuberculous complex. The tuberculin test was positive. A cercarial antigen test was positive on the forty-sixth day and the adult worm antigen skin test was positive on the eighty-fourth day, when it was first used in this case.

Rectosigmoidoscopic studies on the forty-first day after exposure and twenty-sixth day of illness showed generalized edema of the mucosa and multiple punctate hemorrhages. No schistosome eggs were demonstrated in the rectal biopsy specimen. The mucosal changes had disappeared by the fifty-third day when the mucosa assumed a granular appearance. Eggs were not encountered in the rectal mucosa until the sixty-sixth day after exposure.

In view of the increasing weakness and intensification of symptoms, a course of fuadin (stibophen), in intramuscular daily doses of 1.0, 2.0 and 3.0 cc., followed by 3.0 cc. on alternate days, for a total of 78 cc., was initiated on the forty-fifth day after exposure and nineteenth day of illness. Ten days after the initiation of therapy the liver had increased in size and the eosinophil levels had reached 15,168 per cu. mm. of blood. The spiking fever disappeared after nineteen days of therapy, with marked improvement in appetite and a 6 pound weight gain in five weeks. Schistosome eggs disappeared from the stools twenty-three days after the initiation of therapy. The liver and spleen gradually decreased in size, being no longer palpable after the twentieth week.

Seven months after infestation the liver and spleen were of normal size, the stools showed occasional S. mansoni eggs, and the rectal biopsy specimen showed a pseudotubercle with a living egg. Fifteen months after infection neither the liver nor the spleen were palpable, the stools failed to reveal eggs, the hemoglobin was 9.8 gm. per cent, the white blood cell count 5,900, and the eosinophils 1,154 per cu. mm. of blood. Skin tests with cercarial, adult worm and egg antigens were positive. A two-year follow up shows that the patient is in good health. However, live S. mansoni eggs were recovered from the stools and rectal mucosa.

Pathogenesis. There was no correlation between the frequency and duration of exposures and the incidence or severity of the earliest cutaneous manifestations. Likewise, no correlation could be established between the incubation period and the severity of symptoms on the one hand, and the duration of the acute phase of the disease on the other. Thus the shortest incubation (twenty-one days) was observed after a single exposure, and the longest (forty-two days) after several consecutive exposures. The incubation period after repeated consecutive exposures averaged twenty-nine days, and after single exposures, twenty-seven days. The shortest as well as the longest clinical courses were observed among those who had been repeatedly infected (seventeen and seventy-four days respectively). The average duration of this phase after several infections (35.5 days) approached that which followed only one exposure (34.4 days).

The low incidence (33.3 per cent) of the earliest cutaneous manifestations remains unexplained. These phenomena were invariably absent among those exposed for as long as twenty-five consecutive days, while evident in three of the five patients with only one exposure. An analysis of the data would indicate that the degree of infection of the contaminated stream is not the only factor governing the severity of the earliest cutaneous manifestations in Manson's schistosomiasis. Our findings would support other than an allergic causation, and substantiate observations on the poor antigenic

response of the S. mansoni cercariae as compared with avian schistosomiasis.[29,30] It seems that the appearance of early skin changes is not an evidence of massive infection, nor is it governed by previous exposures within a reasonable period (sixteen to twenty-five days) for the development of sensitivity to the allergen of S. mansoni cercariae. This observation may also explain the invariable absence of clinical manifestations during the periods of invasion and incubation.

TABLE III

INFLUENCE OF REPEATED EXPOSURES ON EARLY CUTANEOUS MANIFESTATIONS, INCUBATION PERIOD AND DURATION OF THE FEVER OF ACUTE MANSON'S SCHISTOSOMIASIS

Case	Exposures	Cutaneous Manifestations	Incubation Period (days)	Duration of Fever (days)
3	3	No	30	30
5	16	No	28	74
6	25	No	26	29
7	24	No	27	23
8	24	No	26	38
9	24	No	26	38
10	Several	Yes	42	17

The importance of the degree of infestation of the stream in relation to the length of the incubation period is well illustrated by the seven patients that were repeatedly exposed. Thus four otherwise healthy brothers, aged eight to sixteen years, had twenty-four to twenty-five consecutive exposures leading to incubation periods of twenty-six to twenty-seven days, and a duration of the acute phase ranging from twenty-three to thirty-eight days. By the same token three to sixteen exposures to other streams were followed by incubation periods of twenty-eight to forty-two days and febrile illnesses of seventeen to seventy-four days. (Table III.)

Although the variations in the incubation period were less evident among those with only one exposure, the variability in duration of the acute phase was as remarkable as in those with repeated infections. Thus the incubation period varied from twenty-one to thirty days, the duration of the acute phase from twenty-one to sixty-three days. While an incubation period of twenty-one days led to an acute illness of thirty-three days, and that of thirty to an illness of twenty-six, another of twenty-eight days resulted in a prolonged acute phase of sixty-three days. The unexpected higher incidence of mild cutaneous manifestations among this group of patients remains unexplained. (Table IV.)

Considering that the clinical course of the acute phase of the disease remains unaltered by early institution of specific therapy it appears that the severity and duration of the earliest systemic manifestations of acute schistosomiasis were mainly governed by the capacity of the defensive mechanisms of the host. It must be emphasized that pulmonary manifestations were conspicuously absent during the period of invasion and incubation, that there were no recognizable prodromata, and that the onset was notable for its explosiveness with generalized and systemic clinical manifestations rather than specific involvement of any particular organ or system.

TABLE IV

INFLUENCE OF A SINGLE EXPOSURE ON EARLY CUTANEOUS MANIFESTATIONS, INCUBATION PERIOD AND DURATION OF THE FEVER OF ACUTE MANSON'S SCHISTOSOMIASIS

Case	Approximate Duration of Exposure	Early Cutaneous Manifestations	Incubation Period (days)	Duration of Fever (days)
1	½ hour	No	28	63
2	a few seconds	Yes	28	24
3	1 hour	No	28	21
11	¾ hour	Yes	30	26
12	10 minutes	Yes	21	33

The spontaneous and complete disappearance of the pulmonary roentgenologic alterations lends support to the view that eosinophilic infiltration is the underlying cause.[31] These were more prominent during the earliest stage of the disease when eosinophilic leukocytosis was at maximum levels and was unaltered by fuadin (stibophen) or arsenical therapy.[32] In one instance signs of bronchospasm simulating bronchial asthma persisted intermittently for several months after the institution of therapy.

It must be emphasized that the clinical manifestations were initiated with maturation and oviposition of the infesting parasite, as evidenced by the almost constant finding of ova in the stools and/or rectal mucosa within the first forty days after exposure and three to thirty-four days (average fifteen days) after onset. At this time there was definite evidence of intestinal

and hepatic disturbances as demonstrated by the changes in the rectal mucosa and the abnormal liver function tests.

CASE REPORTS

CASE 3: T. G., a fourteen-year old white Puerto Rican boy, was admitted to the San Juan City Hospital on Nov. 14, 1950, stating that on his arrival from New York he bathed for three consecutive days in a stream known to be heavily infested with S. mansoni. He remained asymptomatic until thirty days after his initial exposure when shaking chills, fever, headache, profuse diaphoresis, cough, generalized body aches and pains, weakness and lassitude, nausea and vomiting, crampy abdominal pains, severe watery diarrhea with tenesmus of one-week duration, dry cough and purpuric rash in the lower extremities suddenly developed and there was rapidly progressive weight loss in spite of good appetite. On the thirty-eighth day after exposure S. mansoni ova were found in the stools. On the forty-second day the leukocyte count was 18,550 per cu. mm. with 42 per cent eosinophils. X-rays of the chest were reported as normal on the forty-first day. The progression of symptoms required his hospitalization sixty days after exposure and thirty days after onset.

He was well developed and nourished, acutely ill and pale with temperature of 100°F., pulse 88 and respirations 22 per minute, and blood pressure of 108 mm. Hg systolic and 72 diastolic. There was generalized lymphadenopathy, moderate splenomegaly and hepatomegaly and extensive purpuric rash in the lower extremities.

From the sixth to the twelfth week of illness his hemoglobin values ranged from 10.7 to 14.5 gm. per cent; leukocyte counts from 11,000 to 18,600 per cu. mm. with 26 to 42 per cent eosinophils; normal serum albumin and globulin; negative cephalin flocculation and bromsulphalein tests, and sedimentation rate of 32 mm. in one hour. The platelet count, the bleeding, clotting and clot retraction times, and the serum bilirubin were normal. Stool and blood cultures for shigella and salmonella as well as serologic tests for typhoid, paratyphoid, typhus, brucellosis and heterophil antibodies were negative. No amebas were observed in the stools. There was increased capillary fragility and prolongation of the prothrombin time to twenty-six seconds (control fifteen seconds) which reverted to normal after the intramuscular administration of vitamin K. An x-ray of the chest on the fiftieth day revealed slight accentuation of the bronchovascular markings without evident parenchymatous alterations.

He was afebrile by the sixtieth day. The purpuric rash disappeared on the sixty-first day. By the seventy-fourth day the spleen and liver were within normal in size when a course of fuadin (stibophen) was given in intramuscular daily doses of 1.5, 3.0 and 5.0 cc., followed by 5 cc. on alternate days for a total of 60 cc. On his return to New York he showed progressive weight gain and marked improvement in his general health. However, three years after infestation living schistosome eggs were recovered from his stools.

CASE 10: B. J., a sixteen-year old Puerto Rican mulatto boy, was admitted to the San Juan City Hospital on June 3, 1954, stating that on April 14, after one of several consecutive exposures to contaminated streams in an endemic area, generalized pruritus and urticaria developed which lasted for two hours. Forty-two days later chilly sensations; high, spiking fever with profuse diaphoresis on defervescence; nausea and vomiting; watery diarrhea and colicky abdominal pain; generalized body aches and pains accompanied by weakness and frontal headache and dry cough developed and there was a rapid weight loss of 18 pounds. The persistence and intensification of symptoms prompted his admission on the fiftieth day after infestation and eight days after onset.

On admission he was stuporous and markedly dehydrated, with a temperature of 105°F., pulse 110 and respirations 26 per minute, and blood pressure 100 mm. Hg systolic and 60 diastolic. Physical examination revealed generalized lymphadenopathy, infected and hypertrophied tonsils and diffuse abdominal tenderness.

Laboratory findings from the eighth to thirteenth week after infestation revealed a mild normochromic anemia; white blood cell counts ranging from 9,400 to 17,100 per cu. mm. with eosinophil counts of 1,900 to 5,000; serum albumin of 2.9 to 3.9 gm. per cent; and globulins of 2.7 to 3.0 gm. per cent; positive cephalin flocculation test during the tenth and eleventh weeks; normal thymol turbidity and bromsulphalein excretion test; accelerated sedimentation rate, and S. mansoni ova in stools and rectal mucosa. Repeated blood and stool cultures were negative for pathogens. The Widal and Weil-Felix agglutinations were negative, and no amebic, malarial or filarial parasites were present. The stools showed eggs of T. trichiura and N. americanus. The serum bilirubin, alkaline phosphatase and prothrombin time were within normal. The urinalyses, serologic tests for syphilis and x-rays of the chest were negative. The skin test with cercarial antigen was positive but those with the adult worm and egg antigens were negative on the seventy-second and one-hundred forty-seventh day after exposure.

There was clinical improvement under supportive therapy, with disappearance of the diarrhea on the fifty-second day. The spiking fever was unaltered by oral doses of 2 gm. of chloramphenicol for three days. It was not until the fifty-ninth day that the temperature reached normal levels, but daily spikes of fever as high as 104.5°F. reappeared on the sixty-second day with spontaneous and persistent remission on the sixty-sixth day after exposure. The liver was felt 2 cm. below the costal border on the fifty-second day. It

gradually decreased in size, reaching normal by the hundredth day after exposure.

A course of 90 cc. of fuadin (stibophen) was started on the one-hundred fourth day, in daily intramuscular doses of 1.5, 3.0 and 5.0 cc., followed by 5.0 cc. on alternate days. The noticeable improvement prior to treatment was now more evident, and despite positive stools for live eggs of S. mansoni 147 days after exposure, he has had an 18 pound weight gain and the liver appeared normal in size.

CASE 12: F. R., a thirteen year-old, white Puerto Rican boy, was admitted to the San Juan City Hospital on July 21, 1954, stating that on June 5, 1954, he went bathing for ten minutes in a stream known to be heavily infested with S. mansoni. He had generalized itching and urticaria for one and one-half hours immediately following exposure. Twenty-one days after the exposure chills, fever, diaphoresis, dry cough, frontal headache and generalized body aches and pains developed. These symptoms subsided spontaneously in eight days, to reappear six days later with abdominal pain, tenesmus, watery diarrhea, puffiness of the eyelids and face, and a rapid weight loss of 17 pounds. The persistence and intensification of these symptoms forced his hospitalization on the forty-sixth day after infestation and fifteenth after onset.

The patient was an acutely ill, poorly nourished, dehydrated boy, in moderate distress, and with a temperature of 101.2°F., pulse 132 and respirations of 24 per minute, and blood pressure of 100 mm. Hg systolic and 80 diastolic. Physical examination disclosed generalized lymphadenopathy, injected pharynx and a soft liver and spleen extending 2 cm. below the costal borders.

Laboratory findings from the seventh to eleventh weeks after infestation revealed hemoglobin values of 9.7 to 10.2 gm. per cent; leukocyte counts of 9,200 to 16,500 per cu. mm. with eosinophils of 4,200 to 10,200; high serum globulin; positive cephalin cholesterol flocculation test until the tenth week; thymol turbidity of 8.3 units; bromsulphalein retention of 36 per cent by the eighth week and of 6 per cent by the eleventh; accelerated sedimentation rate, and S. mansoni ova in the stool and rectal mucosa. Stool and blood cultures were negative for pathogens; the urinalyses were normal, and neither malaria nor ameba were seen. The blood Wassermann test was doubtful. X-rays of the chest on the fifty-third day after infestation revealed increased vascular markings. Rectosigmoidoscopic examination on the forty-seventh day showed injected, edematous mucosa with multiple tiny, yellowish granules, pinpoint hemorrhages, and small superficial ulcerations in the rectum and sigmoid. A rectal biopsy specimen failed to show eggs of S. mansoni until two days later (forty-ninth day). On the seventy-fourth day the mucosa was moderately edematous, granular, hyperemic and showed a few pin-point hemorrhages. The skin test was positive with the cercarial antigen, and negative with the adult worm and egg antigens on the sixty-first day.

Although the remittent fever and diarrhea disappeared spontaneously on the fifty-fourth day, soft stools persisted until the sixty-sixth day after infestation. Fuadin (stibophen) was started on the fifty-fourth day in daily intramuscular doses of 1.5, 3.0 and 5.0 cc., followed by 5.0 cc. on alternate days for a total of 100 cc. Treatment was followed by rapid clinical improvement, a weight gain of 6½ pounds and disappearance of splenic enlargement. However, eosinophilic leukocytosis and hepatomegaly persisted for twenty-eight weeks after infestation in spite of negative stools and rectal biopsy specimen for live eggs of S. mansoni.

Hepatic Alterations. Hepatomegaly, ranging from 1 to 3 cm. below the right costal margin in the mid-clavicular line, was encountered in eleven instances on admission. This finding was not evident until the sixty-second day in a patient admitted thirty-two days after exposure. The organ was soft in eleven, firm in one and tender in five; none showed unusual hardening or palpable lobulations. The hepatomegaly was invariably accompanied by a generalized, discrete lymphadenopathy and by splenomegaly in ten instances.

The hepatic enlargement was no longer evident by the twenty-seventh week in seven patients, and of these six received early therapy with fuadin (stibophen). Persistence of hepatomegaly for fifteen months after exposure, without clinical evidence of liver damage after five years of study, was noted in one instance. Two patients showed persistent hepatomegaly and splenomegaly for two and three years, respectively, after early treatment with 60 cc. of fuadin. Hepatomegaly (3 cm.) was observed twenty-eight weeks after infection in two patients in spite of the administration of 100 cc. of fuadin (stibophen) from early in the course of the disease. The significance of the increased hepatic enlargement, concomitant with intensification of the eosinophilic leukocytosis, observed in seven patients after initiation of therapy remains unassessed. The transitory increase in hepatic tenderness may be attributed to acute stretching of Glisson's capsule.

While invariably abnormal during the fifth to the eighth week after exposure, the cephalin cholesterol flocculation test generally reverted to normal within the twenty-first to the twenty-third week. (Table v.) The total serum proteins

were altered in six instances. Plasma albumin was decreased (below 3 gm. per cent) in three cases. Hyperglobulinemia (values above 2.5 gm. per cent) was invariably present, with values as high as 6.3 gm. per 100 cc. of blood in case 1, exceeding 3 gm. in ten, and 4 gm. per cent in five, during the first seven to twelve weeks after exposure. This phenomenon was evident for as long as twenty-one to twenty-three weeks, respectively, in two instances. Electrophoretic studies in six patients showed significant increases in the gamma fraction. A bromsulphalein retention of over 5 per cent in forty-five minutes was observed in six of ten patients during the fifth and ninth week after infection but in only three of these was it abnormal by the tenth to the twelfth week. The thymol turbidity test (over 5 units) was abnormal in all eight patients tested, persisting until the seventh to the fifteenth week after infection. (Table VI.) Eleven of the twelve patients showed normal prothrombin times. In one, prolongation to twenty-six seconds, with a control of fifteen, was effectively remedied with intramuscular vitamin K. Three patients showed false positive serologic tests for syphilis. The data of the liver function tests are summarized in Table VII.

Needle liver biopsies were performed in three instances. The earliest was obtained sixty-eight days after exposure and forty days after onset of the illness. The hepatic parenchyma was well preserved but showed areas of diffuse eosinophilic and lymphocytic infiltration, more marked in the portal spaces. Since oviposition had al-

TABLE V
ABNORMALITIES OF SERIAL CEPHALIN FLOCCULATION TESTS

	Weeks after Exposure						
	5 to 6	7 to 9	10 to 12	13 to 15	16 to 18	19 to 20	21 to 23
Number of cases	3	9	11	8	7	6	6
Number abnormal ..	2	8	10	7	7	3	0

TABLE VI
SUMMARY OF LIVER FUNCTION TESTS

Case	Serum Total Protein (gm. %)	Serum Albumin (gm. %)	Serum Globulin (gm. %)	Cephalin Flocculation Test	Thymol Turbidity Test	Bromsulphalein Retention (%)	Prothrombin Time	Serologic Test for Syphilis	Serum Bilirubin
1	9.9	3.6	6.3	4+	....	4	Normal	Positive	.....
2	9.0	4.1	4.9	4+	....	4	Normal	Negative	Normal
3	6.6	3.8	2.8	0	....	4	Abnormal	Negative	.....
4	7.3	4.4	2.9	2+	....	..	Normal	Negative	Normal
5	8.6	4.1	4.5	4+	16.0	..	Normal	Negative	Normal
6	6.2	3.0	3.2	4+	10.0	8	Normal	Positive	.....
7	6.2	2.8	3.4	4+	18.6	6	Normal	Negative	.....
8	6.4	3.0	3.4	3+	10.7	6	Normal	Negative	.....
9	6.0	2.8	3.2	4+	17.2	12	Normal	Negative	.....
10	5.9	2.9	3.0	4+	7.9	2	Normal	Negative	Normal
11	8.6	4.4	4.2	3+	8.9	16	Normal	Negative	Normal
12	8.6	4.4	4.2	4+	8.3	36	Normal	Doubtful	.....

Note: 25 seconds with a control of 15 seconds.

TABLE VII

RESULTS OF THERAPY WITH VARIABLE DOSES OF FUADIN (STIBOPHEN)

Case	Age (yr.)	First Treatment (days after infection)	Amount (cc.)	Early Results	Duration of Fever (days)	Second Treatment (days after infection)	Amount (cc.)	Early Results	Delayed Results	Follow-up	Final Clinical Evaluation
1	14	111	40	Failure*	63	161	40	Success† (7 mo.)	Failure (3 yr.)	Success (5 yr.)	Success Clinically well
2	15	112	40	Failure	24	154	40	Failure (5 mo.)	Failure (3 yr.)	Success (5.5 yr.)	Success Clinically well
3	14	79	60	Failure	30	...	..	Failure (9 mo.)	Failure (1.5 yr.)	Failure (3 yr.)	Failure Clinically well
4	13	70	60	Failure	21	360	60	Failure (1 yr.)	Failure (1.5 yr.)	Failure (2 yr.)	Failure‡ Hepatomegaly Splenomegaly
5	16	115	60	Failure	74	270	60	Failure (9 mo.)	Failure (2 yr.)	Failure (3 yr.)	Failure Hepatomegaly Splenomegaly
6	12	45	99	Success (14 days)	29	...	..	Success (7 mo.)	Failure (1.5 yr.)	Failure (2 yr.)	Failure Clinically well
7	10	44	99	Success (33 days)	23	...	..	Success (7 mo.)	Failure (1.5 yr.)	Failure (2 yr.)	Failure Clinically well
8	9	45	78	Success (23 days)	38	...	..	Success (7 mo.)	Failure (1.5 yr.)	Failure (2 yr.)	Failure Clinically well
9	8	45	78	Success (17 days)	38	...	..	Failure (7 mo.)	Failure (1.5 yr.)	Failure (2 yr.)	Failure Clinically well
10	16	104	90	Failure	17	...	..	Failure (147 days)	.....	.....	Failure Clinically well
11	14	58	100	Success (22 days)	26	...	..	Success (29 wk.)	.....	.....	Failure Hepatomegaly
12	13	54	100	Success (13 days)	33	...	..	Success (28 wk.)	.....	.....	Failure Hepatomegaly

* Determined by repeatedly positive stools and/or rectal biopsy specimens for live S. mansoni ova or by clinical signs of active Manson's schistosomiasis.

† Determined by repeatedly negative stools and/or rectal biopsy specimens for live ova of S. mansoni.

‡ In spite of 60 cc. of fuadin (stibophen) administered twenty-one months, and 180 cc., two years after exposure.

ready begun, it is reasonable to believe that the limited portion of hepatic tissue was obtained from an area remote from eggs and pseudotubercles. The eosinophilic infiltration indicates that the allergic response is not limited to the areas surrounding the source of allergen (worms and/or eggs) but is a generalized reaction like that occurring in infiltrative eosinophilia[31] (Case 5).

Biopsy specimens, eighty and 115 days after infection (fifty-two and eighty-seven days after onset, respectively) showed an unaltered parenchyma but numerous pseudotubercles, many of them with eggs in their center and with moderate eosinophilic and lymphocytic infiltration about the periphery. The diffuse eosinophilic infiltration observed earlier had disappeared and at this time the reaction was well localized around the source of allergen. (Cases 1 and 5.)

Biopsy specimens taken fifteen and eighteen months after exposure, and after treatment with fuadin (stibophen), revealed numerous pseudotubercles with egg shells in their center, surrounded by a slight eosinophilic and lymphocytic infiltration. There was a slight increase in portal fibrosis with formation of pseudolobules in one of the sections. The liver parenchyma was well preserved, aside from slight fatty changes, and there was no proliferation of bile ducts.

CASE REPORTS

CASE 1: G. R., a fourteen year old white Puerto Rican boy, was admitted to the San Juan City Hospital on July 28, 1948, stating that he had gone swimming in a small infected stream in the center of the island. This was his first and only exposure. He felt well until twenty-eight days later when puffiness

of the eyelids and face, weakness, generalized body aches and pains, anorexia, chills, fever, crampy, abdominal pains, bloody and mucoid diarrhea with tenesmus, nausea and vomiting and non-productive cough suddenly developed and there was a rapid weight loss of 10 pounds. The progression and intensification of these symptoms prompted his hospitalization on the fifty-fourth day after infestation.

He was poorly nourished and acutely ill, with a temperature of 100°F., pulse of 104 and respirations of 24 per minute, and a blood pressure of 100 mm. Hg systolic and 70 diastolic. Physical examination disclosed generalized discrete lymphadenopathy (cervical, axillary, inguinal, epitrochlear); moist rales over both lung bases; a firm, non-tender liver extending 1 cm. below the right costal margin, and an increased area of splenic dullness.

From the eighth to the twenty-third week after exposure he showed normal hemoglobin and red cell values; leukocyte counts varied from 14,300 to 20,500 per cu. mm. with 35 to 80 per cent eosinophils; normal serum albumin with definite hyperglobulinemia ranging from 3.75 to 6.25 gm. per cent; positive cephalin cholesterol flocculation test until the seventeenth week; normal bromsulphalein test; accelerated sedimentation rate, and S. mansoni ova first seen in the stools on the seventy-fourth day and in the rectal mucosa, on the one hundred fifth day. Repeated blood and stool cultures were negative for bacterial pathogens; blood smears were negative for malaria; no E. histolytica were found either in stools or rectal mucosal scrapings; there was no serologic evidence of typhoid, paratyphoid, typhus fever or brucellosis, and cold hemagglutinins and heterophile antibodies were absent. A false positive Wassermann test was observed in the early phases of the disease; the prothrombin time, icterus index, urinalysis, urinary urobilinogen, and the glucose and galactose tolerance tests were negative. The bone marrow showed increased eosinophils (27 per cent) but was otherwise normal. The electrocardiogram was within normal limits. X-ray of the lungs on the fifty-sixth day after exposure revealed bilateral basal bronchopneumonia. A needle biopsy specimen of the liver on the eightieth day after exposure and fifty-second of illness showed eosinophilic infiltration of the portal spaces with several pseudotubercles containing eggs of S. mansoni with embryos. There was no evidence of fibrinosis. (Fig. 1.)

The nausea and vomiting disappeared on the sixty-first day and the non-productive cough and roentgenologic pulmonary findings on the sixty-seventh day after infestation. On the eightieth day evanescent pulmonary wheezing occurred. The low-grade fever persisted until the ninety-ninth day. Continuous diarrhea persisted until the fifty-first day, and intermittently until the 113th.

A course of fuadin (stibophen) in doses of 1.5, 3.5, 5.0 daily, followed by 5.0 cc. on alternate days for a total of 40 cc., was administered intramuscularly from the 111th to the 144th day. A transitory painful hepatic enlargement to 5 cm. below the right costal margin, concomitantly with an increase in the leukocyte count and eosinophilia, followed the first injection. However, within a few days his appetite improved, with a weight gain of 4 pounds in the following two weeks. A second course of fuadin (stibophen) during the twenty-third to twenty-sixth weeks led to further general improvement.

The leukocyte count was 10,850 per cu. mm. with 12 per cent eosinophils; serum albumin 5.7 and globulin 1.8 gm. per cent; the cephalin cholesterol flocculation test was negative, and stools were negative for S. mansoni eggs fifteen months after exposure. Although the liver was normal in size, a needle biopsy specimen revealed portal fibrosis and numerous schistosomal pseudotubercles.

Five years after exposure he is well developed and nourished, participates freely in athletics and, although biopsy of the liver has not been repeated, he does not present any clinical or laboratory abnormality indicative of active schistosomiasis.

CASE 2: P. C., a fifteen-year old white Puerto Rican boy, was admitted to the San Juan City Hospital on July 14, 1948, stating that thirty-two days prior admission, while trying to ford a small stream heavily infested with S. mansoni, he slid on a mossy stone, soaking his feet in the contaminated water. This first and only exposure was soon followed by itching of the feet and ankles during half an hour. His good general health remained unaltered until twenty-eight days after infestation when chills, fever, profuse perspiration, general malaise, body aches and pains, arthralgia, weakness, anorexia, headache, a dry cough, nausea and vomiting, crampy abdominal pains and watery diarrhea with tenesmus suddenly developed and there was rapid weight loss.

He was acutely ill and poorly nourished, with a temperature of 99°F., pulse rate of 108 and respirations of 24 per minute and the blood pressure was 100 mm. Hg systolic and 70 diastolic. Physical examination disclosed enlargement of cervical lymph nodes, dullness over both lung bases posteriorly with numerous moist rales and diffuse abdominal tenderness.

From the sixth to the twenty-second week after infestation the hemoglobin and red cell values remained normal; the leukocyte counts ranged from 7,900 to 28,900 per cu. mm. with 19 to 64 per cent eosinophils; the serum albumin was normal in the presence of hyperglobulinemia (4.1 to 5.6 gm. per cent). The cephalin cholesterol flocculation test was strongly positive until the twentieth week. The bromsulphalein and galactose tolerance tests, and the serum bilirubin were invariably within normal and the formol gel test was positive. S. mansoni eggs appeared in the stools on the sixtieth, and in the rectal mucosa on the ninety-first day after infestation. Stool

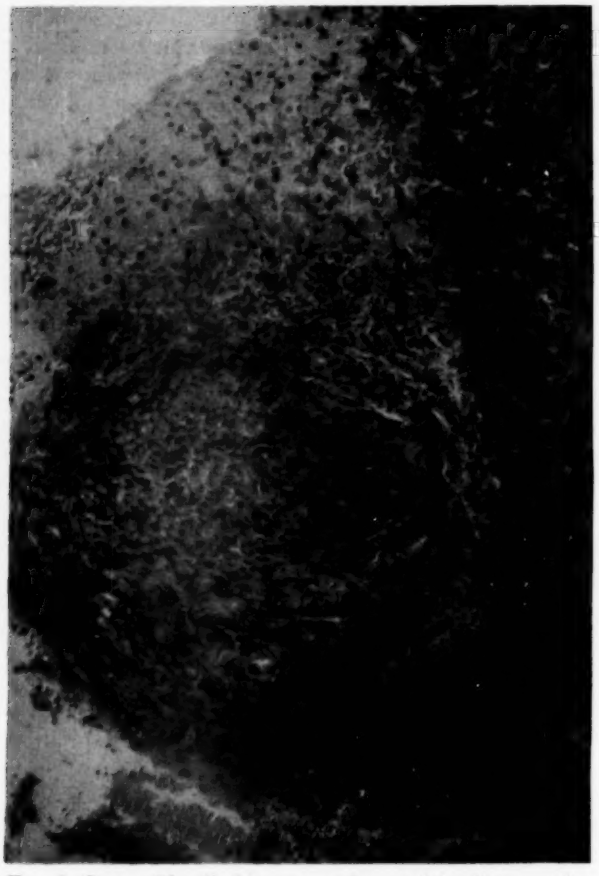

FIG. 1. Case I. Needle biopsy specimen of the liver on the eightieth day after infestation and fifty-second after onset, showing eosinophilic infiltration of the portal spaces and a pseudotubercle; original magnification, × 360.

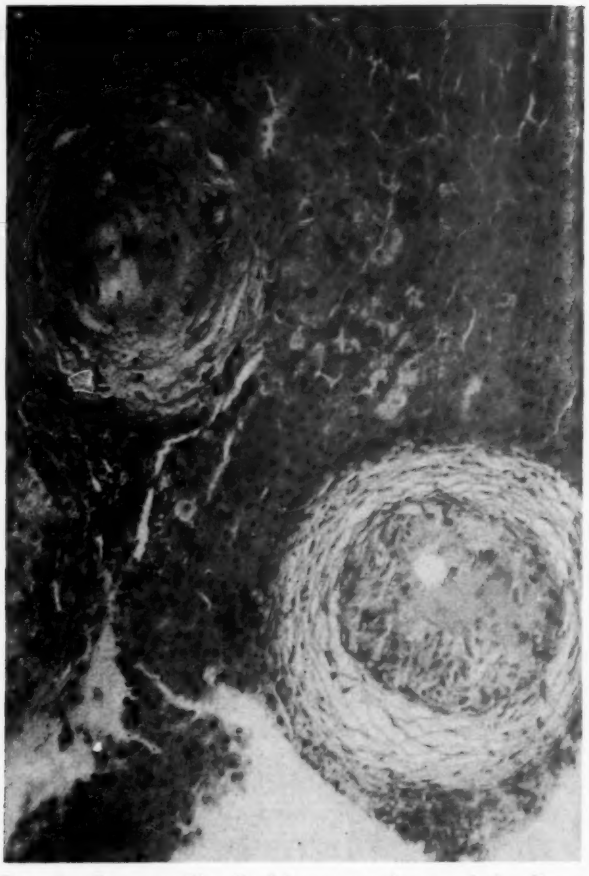

FIG. 2. Case II. Needle biopsy specimen of the liver eighteen months after exposure and fourteen months after therapy showing two pseudotubercles and scanty eosinophilic infiltration. No portal fibrosis was observed; original magnification, × 360.

and blood cultures were negative for bacterial pathogens, and there was no diagnostic serologic evidence for typhoid, paratyphoid, typhus fever or brucellosis. Cold agglutinins and heterophil antibodies were lacking and the Kahn test was negative. Neither malarial parasites nor E. histolytica were present. Repeated urinalyses were normal. The electrocardiogram was normal. Roentgenologic evidence of bilateral bronchopneumonia, discovered on the thirty-seventh, persisted until the fifty-fourth day after exposure.

A daily spiking fever to 104°F., non-productive cough and diarrhea with tenesmus persisted until the fifty-second day. By the sixty-second day the soft and tender liver was palpated 2 cm. below the right costal margin. A course of fuadin (stibophen) in daily doses of 1.5, 3.5, followed by 5.0 cc. intramuscularly on alternate days for a total of 40 cc., was initiated on the fourteenth week. Four days after the first injection the liver increased in size, with accompanying increases in the leukocyte and eosinophil counts. By the twentieth week the liver was normal in size, and another course of fuadin (stibophen) of 40 cc. was started on the twenty-second week with rapid disappearance of symptoms and progressive weight gain.

Eighteen months after infestation he had a leukocyte count of 6,950 with 10 per cent eosinophils; serum albumin 4.4 and globulin 3.4 gm. per cent, and the prothrombin time, bromsulphalein and cephalin flocculation tests were normal. A rectal biopsy specimen showed dead S. mansoni eggs. A needle biopsy specimen of the liver revealed several pseudotubercles with centrally located egg shells and scanty eosinophilic and neutrophilic infiltration. (Fig. 2.)

Forty-two months after infestation he was readmitted with anicteric leptospirosis. He made a prompt and uneventful recovery. At this time the serum globulin was 2.2 gm. per cent and albumin 4.8 gm. per cent and live S. mansoni eggs were found in the stools and rectal biopsy specimen.

Five and a half years after exposure he is well developed and nourished, with no clinical findings indicative of active schistosomiasis. Only dead eggs of S. mansoni were found in the rectal biopsy specimen. There was an eosinophilic count of 1,210 per cu. mm. of blood.

CASE 5: R. P., a sixteen-year old white Puerto Rican boy, bathed for sixteen consecutive days in a heavily infested stream without immediate skin manifestations. Twenty-eight days after the first exposure he was suddenly taken with chills, high fever, profuse perspiration, weakness, dry cough, generalized arthralgia and muscular aches and pains, transitory puffiness of the eyelids and anorexia. The persistence of these symptoms and the development of severe watery diarrhea required his hospitalization on the sixty-second day after exposure and thirty-fourth day after onset.

He appeared moderately ill with a temperature of 103°F., pulse 110 and respirations 20 per minute, and blood pressure of 120 mm. Hg systolic and 80 diastolic. There was generalized, non-tender lymphadenopathy; a tender liver felt 3 cm. below the costal margin, and a firm non-tender spleen felt 2 cm. below the costal border.

The laboratory findings from the tenth to the twentieth week revealed normal hemoglobin values; white blood cell counts from 7,100 to 11,000 per cu. mm. with 2,200 to 5,500 eosinophils; hyperglobulinemia with normal serum albumin; positive cephalin cholesterol flocculation test and accelerated erythrocyte sedimentation rate. Schistosome ova were found in the stools on the sixty-third day and in the rectal mucosa on the ninety-sixth day after exposure. The serum bilirubin, prothrombin time, urinalysis and urinary urobilinogen were repeatedly normal. Stool and blood cultures, Widal, Weil-Felix, brucella agglutinations and Kahn test were negative. Neither amebas nor malarial parasites were discovered. The serum alkaline phosphatase was 5.2 Bodansky units and the thymol turbidity varied from 5 to 16 units. The bone marrow was normal apart from increased eosinophilic elements. The x-ray of the chest was normal.

On the sixty-eighth day after exposure and fortieth of illness a needle biopsy specimen of the liver revealed areas of diffuse lymphocytic and eosinophilic infiltrations, mainly in the portal spaces. (Fig. 3.) Another liver biopsy on the one hundred-fifteenth day after exposure and eighty-seventh day after onset showed pseudotubercles containing schistosome eggs.

Spontaneous decline of the daily spiking fever was noted on the eighty-seventh day but low grade elevations persisted until the one hundred second day after exposure. Evanescent pulmonary signs (wheezes and rhonchi) were repeatedly detected during hospitalization. The liver was felt 5 cm. below the right costal margin on the 84th day.

Treatment with fuadin (stibophen) in daily intramuscular doses of 1.5, 3.0 and 5.0 cc., followed by 5.0 cc. on alternate days for a total of 60 cc., was initiated on the 115th day. Treatment was followed by rapid clinical improvement. Six months after infestation the liver and spleen remained moderately enlarged, and the cephalin cholesterol flocculation test was positive for seven months. Live S. mansoni eggs were present in the rectal mucosa for nine months after treatment, when another course of 60 cc. of fuadin (stibophen) was initiated by the method already outlined. Normal eosinophil levels were not attained until the tenth month after exposure.

Three years after exposure moderate splenomegaly and hepatomegaly persist, and live eggs of S. mansoni have been repeatedly seen in biopsy specimens of the rectal mucosa.

Treatment. The diagnosis was established during the first week of hospitalization by the demonstration of the typical S. mansoni eggs in the stools in eight, and in rectal biopsy specimens in one instance, within an average of forty-five days after exposure and 16.4 days after onset. In three cases eggs were demonstrated in the stools or rectal biopsy specimens within twenty to thirty-three days after hospitalization (an average of sixty-seven days after exposure and thirty-nine of illness).

Treatment was initiated forty-four to one hundred fifteen days after exposure (average 73.5 days), seventeen to eighty-seven days after onset of symptomatology (average forty-six days). The first courses of fuadin (stibophen) varied from 40 to 100 cc. Eight patients received the drug in daily intramuscular doses of 1.5, 3.5 and 5.0 cc. followed by 5.0 cc. on alternate days for a total of 40 to 100 cc.; 2, in daily doses of 1.0, 2.0 and 3.0 cc. followed by 3.0 cc. on alternate days for six doses, and with further increases to 5.0 cc. every other day for a total of 99 cc. In the two youngest patients the maximal dose was limited to 3.0 cc. on alternate days for a total of 78 and 78.5 cc., respectively. Four patients received a second course of 40 to 60 cc. of the drug.

In nine cases treatment was instituted after the disappearance of fever; in the other three fuadin (stibophen) failed to alter its duration. Increases in temperature were observed from the seventh to the nineteenth day of treatment in a patient who was previously afebrile. Transitory low grade fever was frequently observed during the earliest phases of treatment. Diarrhea disappeared prior to therapy in ten instances (average duration 25.5 days). In two it disappeared on the first and twelfth day of treatment, respectively.

Most of the patients claimed improvement in appetite, rapid disappearance of weakness and lassitude, and weight gain of 2 to 13.5 pounds (average 7.3 pounds) within two to seven weeks

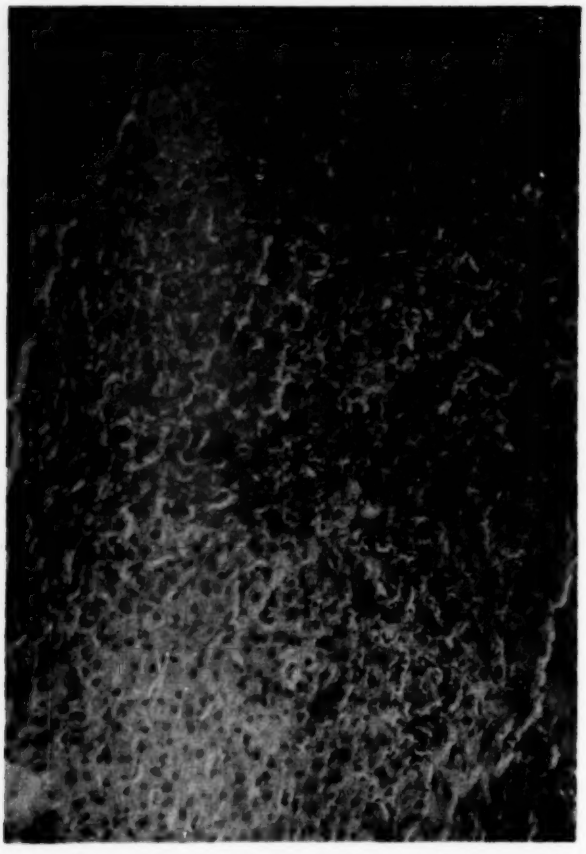

FIG. 3. Case V. Needle biopsy specimen of the liver on the sixty-eighth day after exposure and fortieth of illness showing diffuse eosinophilic and lymphocytic infiltration mainly in the portal spaces. The liver cells are well preserved; original magnification, × 360.

after the initiation of therapy. In one case the liver was normal in size prior to treatment; in six it was no longer palpable by the twenty-seventh week, while in five hepatomegaly persisted after therapy.

The effect of treatment on the invariably present hypereosinophilia was carefully observed in eight instances. It remained unaltered for three to fourteen weeks after therapy in four; minor to moderate decreases were observed in three, and normal levels were reached in one case. In one instance the hypereosinophilia persisted for over a year in spite of early therapy. Therapy failed to alter the accelerated sedimentation rates in three cases within the first six to twenty-one weeks after exposure.

No significant quantitative alterations were observed in the serum globulins of five patients studied for one to seven weeks after the initiation of treatment. Electrophoretic studies showed persistently increased gamma fractions prior to therapy, with marked decreases after the twenty-first week. In six patients in whom treatment was initiated on the seventh to the eighteenth weeks after exposure the cephalin cholesterol flocculation test was normal by the nineteenth to the twenty-first week. It is emphasized that in four of these return to negative cephalin flocculation test was delayed for six weeks after completion of therapy (average total dose, 88.6 cc.). It remained unaltered during the first two to seven weeks of treatment (average total dose 69 cc.) in those who received treatment within the first forty-five days after exposure.

Live eggs persisted in the stools and rectal mucosa after the termination of an initial course of 40 cc. in 2, 60 cc. in three, and 90 cc. of fuadin (stibophen), in one instance. In the other six patients live S. mansoni eggs had disappeared from the stools and/or rectal mucosa within seventeen and twenty-three days in two patients who received 78 cc. of the drug; within fourteen and thirty-three days in two who received 99 cc., and within thirteen and twenty-two days in two treated with 100 cc. of the drug. Thus the immediate results with total doses in excess of 75 cc. were favorable in respect to cessation of oviposition. It would appear that many of the eggs deposited in the rectal mucosa were rapidly destroyed and the mechanical and local allergic effects were suppressed. However, even during the period of cessation of oviposition the clinical manifestations derived from the explosive hypersensitivity remained unaltered, as exemplified by the variable duration of fever and eosinophilia among those treated on different dates and with different doses of the drug.

It must be emphasized that oviposition may be suppressed for as long as one year by the administration of 40 to 100 cc. of fuadin (stibophen). This emphasizes the essentialness of a long follow-up in the determination of curability. It would seem that the drug is suppressive rather than curative even when administered in heavy dosage.

In the group of initial failures, four patients received another course of 40 to 60 cc. of fuadin (stibophen) six to thirty-eight weeks following the initial treatment. One of these patients failed to show eggs either in the stools or rectal mucosa seven months later, while the other three showed signs of persistent infection after 1.2 to 3 years. All those six patients considered to be therapeutic successes initially later showed evidence either of clinically active schistosomiasis or were found to have live eggs in the stools or

rectal biopsy specimens. The initial therapeutic failures were not affected by a second treatment, and one patient failed to respond to a total of 240 cc. of the drug.

From the analysis of these data it would appear that the infection in acute Manson's schistosomiasis is unaltered by the administration of fuadin (stibophen) in total doses as high as 100 cc. by the methods employed. The chances of curability may not be increased by early institution of therapy with larger doses of the drug. Lower total doses (40 to 60 cc.), even when repeatedly administered, fail to alter the course of the disease. It is evident that 40 to 100 cc. of the drug may not suffice to destroy the adult parasite, to suppress oviposition, or to arrest the pathologic alterations. It must be emphasized that early institution of therapy fails to alter the commonly observed hepatic alterations, as evidenced by persistence and probable progression of the lesions. Spontaneous reversion to normal of the liver function tests may be explained in alternative ways.

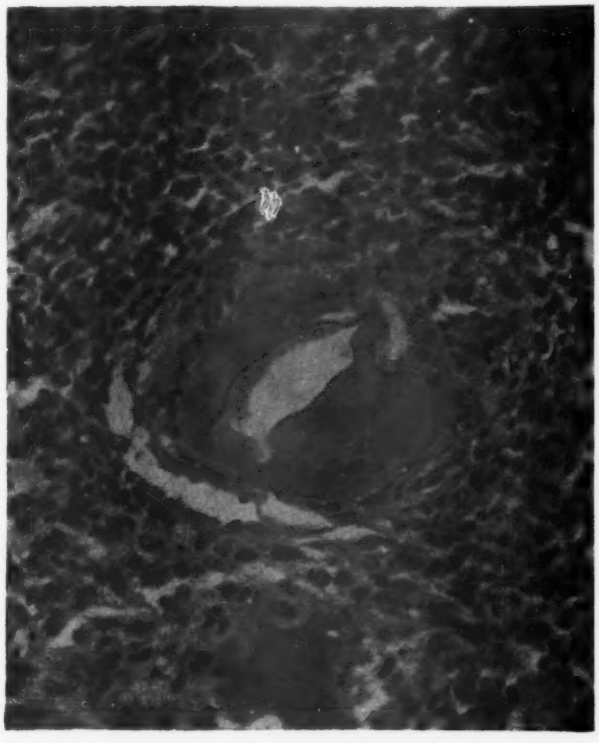

FIG. 4. Case 1. Biopsy specimen of the liver on the eightieth day after exposure and fifty-second of illness showing a typical pseudotubercle containing a schistosome egg. Note the profuse eosinophilic infiltration; original magnification, × 360.

CASE REPORTS

CASE 4: S. P., a thirteen-year old mulatto Puerto Rican boy, was admitted to the San Juan City Hospital on August 9, 1952, stating that twenty-eight days after his first and only exposure in an infected stream he was suddenly taken with shaking chills, spiking fever, profuse perspiration, frontal headache, anorexia, dizziness, generalized weakness, nausea, vomiting, watery diarrhea with tenesmus, and crampy abdominal pain, loss of weight and dry cough. The persistence and intensification of these symptoms prompted his hospitalization on the thirty-fourth day after exposure and sixth day of illness.

The patient was prostrated with a temperature of 102°F., pulse 100 and respirations 20 per minute, and a blood pressure of 90 mm. Hg systolic and 60 diastolic. He presented generalized lymphadenopathy, a moderately enlarged and tender liver, and a barely palpable spleen.

Laboratory findings from the sixth to the tenth week after infestation revealed hemoglobin values of 10.8 to 12.4 gm. per cent; leukocyte counts of 4,700 to 14,700 per cu. mm. with 16 to 42 per cent eosinophils; quantitatively normal serum proteins; doubtful cephalin cholesterol flocculation test; a rapid sedimentation rate and schistosome eggs in the stools. No malarial parasites or amebas were found. The blood and stool cultures, Widal, Weil-Felix and brucella agglutinations were negative. The Kahn test, thymol turbidity test, serum bilirubin, blood cholesterol, and the non-protein and urea nitrogen were within normal limits. The x-ray of the chest was normal. A biopsy specimen of a cervical lymph node revealed a chronic lymphadenitis with moderate eosinophilic infiltration.

The fever disappeared spontaneously on the forty-ninth day after exposure but recurred on the fifty-seventh day, with spikes up to 102.5°F. which persisted until the sixtieth day. The coughing and the nausea and vomiting subsided on the thirty-fourth day, the diarrhea on the thirty-ninth day after exposure.

On the seventieth day he was given a course of 60 cc. of fuadin (stibophen) in daily intramuscular doses of 1.5, 3.0, 5.0 cc. followed by 5.0 cc. on alternate days, with favorable clinical response apart from occasional bouts of bloody diarrhea and epigastric distress which had no relation to food intake.

Eleven months after exposure he had a febrile episode of six days' duration with soft, bloody stools and tenesmus. At this time there was hepatic enlargement to 3 cm. below the right costal border, and the spleen was felt 2 cm. below left costal margin. Both organs were firm and non-tender. Rectosigmoidoscopic studies revealed an erythematous, granular mucosa, with numerous easily bleeding, small and shallow ulcerations. Biopsy specimen of the rectal mucosa demonstrated numerous living and dead S. mansoni eggs. He had a red blood cell count of 4,600,000 with 10.8 gm. per cent of hemoglobin, and a leukocyte count of 7,100 per cu. mm. with 12 per cent

eosinophils, as well as a normal prothrombin time, a normal bromsulphalein test and a negative cephalin cholesterol flocculation test. Repeated blood and stool cultures were negative. No amebas were present in the stools or in material aspirated from ulcers in the rectal mucosa. The Widal, Weil-Felix, brucella and leptospiral agglutinations and complement fixation tests were negative. Barium enema and x-ray of the chest were normal.

Improvement followed supportive therapy with disappearance of ulcers from the rectal mucosa within two weeks of hospitalization. A course of 60 cc. of fuadin (stibophen) in intramuscular doses of 1.5, 3.0, 5.0 cc. daily, followed by 5.0 cc. on alternate days, was initiated one year after exposure, with further clinical improvement.

Twenty-one months after infestation he suffered from mild-epigastric distress and exhibited a firm, non-tender liver palpated 2 cm. below right costal margin, and a firm, non-tender spleen extending 2 cm. below the left costal border. The rectal mucosa was granular and loaded with living and dead S. mansoni eggs. He was given another course of 60 cc. of fuadin (stibophen) by the method outlined. Twenty-five months after infestation he was undernourished and anorexic, and the liver and spleen were felt 2 cm. under the costal margins. At this time he had 12.5 gm. per cent of hemoglobin; a white blood cell count of 9,100 with 891 eosinophils per cu. mm. of blood; the stool and rectal biopsy specimen were loaded with living S. mansoni eggs; the bromsulphalein retention was 2 per cent in forty-five minutes; the prothrombin time, 18.5 seconds (control 13.5 seconds); the serum albumin was 4.4 gm. per cent and the serum globulin, 2.16 gm. per cent; the serum alkaline phosphatase 6.7 Bodansky units and the cephalin flocculation test was negative. Another course of 120 cc. of fuadin (stibophen) was started, following the previous dose schedule, with complete failure as evidenced by positive stools and rectal biopsy specimens three years after exposure.

Case 9: E. A., an eight-year old white Puerto Rican boy, was admitted to the San Juan City Hospital on August 11, 1953, stating that on the twenty-sixth day after the first of twenty-four consecutive exposures in a stream heavily infested with S. mansoni chills, fever, headache, profuse diaphoresis, dry cough, nausea, vomiting, crampy abdominal pains, watery diarrhea with tenesmus and anorexia suddenly developed and there was rapid weight loss. The persistence and progression of these symptoms required his hospitalization thirty-seven days after the initial exposure and eleven days after onset.

On physical exploration he appeared acutely ill and dehydrated with a temperature of 103°F., pulse 120 and respirations, 28 per minute. There was generalized lymphadenopathy, a tender liver palpated 2 cm. below the right costal margin, moderate splenomegaly and mild generalized abdominal tenderness.

Laboratory studies from the sixth and twenty-first week showed hemoglobin values of 9.5 to 11.6 gm. per cent; leukocyte counts of 6,800 to 18,800 per cu. mm. with 900 to 12,400 eosinophils; hyperglobulinemia with quantitatively normal serum albumin; significantly positive cephalin cholesterol flocculation test until the seventeenth week; abnormal thymol turbidity test until the eleventh week; 12 per cent retention of bromsulphalein in forty-five minutes on the sixth week; accelerated erythrocyte sedimentation rates, and S. mansoni ova in the stools on the forty-first day after exposure.

Stool and blood cultures, and serologic tests including Widal, Weil-Felix, brucella agglutinations and the Kahn test, were negative. The urinalysis and prothrombin time were normal. No amebas or malarial parasites were found. The tuberculin test was positive and the X-ray of the chest revealed a healed primary tuberculous complex. Skin tests revealed positive reactions to cercariae and adult worm antigens on the eighty-fourth day after the first exposure. Rectosigmoidoscopic studies the eighth and ninth week after exposure showed generalized edema and granulation of the mucosa and multiple punctate hemorrhages in the lower sigmoid and rectum. Live S. mansoni eggs were repeatedly seen in rectal biopsy specimens.

Daily spiking fever was recorded until the forty-third day after exposure and seventeenth day of illness when it subsided spontaneously, to reappear on the fifty-second to the sixty-fourth day. Fuadin (stibophen), in daily intramuscular doses of 1.5, 2.0 and 3.0 cc. followed by 3.0 cc. on alternate days, was started on the forty-fifth day after exposure for a total of 78.5 cc. The first injections were followed by accentuation of the hepatomegaly and the highest eosinophilic levels recorded in this instance (15,100 per cu. mm.). Improvement in the general symptomatology followed soon after the initiation of therapy, with disappearance of S. mansoni eggs from the stools in seventeen days. The liver and spleen were normal in size, the eosinophil count had reached normal levels (900 per cu. mm.) and he had a 10 pound weight gain by the twenty-third week after exposure.

Seven months after infestation he was clinically well, the liver and spleen were normal in size, but live S. mansoni ova were present in the stools, in spite of negative rectal biopsy specimens. Fifteen months after infestation the liver and spleen were normal, the stools revealed live S. mansoni ova and the hemoglobin was 11.2 gm. per cent, the white blood cell count 8,600 cu. mm. and eosinophils 1,530. Skin tests with cercariae, adult worm and egg antigens were positive. Although clinically well, live S. mansoni eggs are still demonstrated in the stools and rectal biopsy specimen two years after infection.

CASE 11: J. S., a fourteen-year old, white Puerto Rican boy, was admitted to the San Juan City Hospital on August 1, 1954, stating that thirty days after his first and only exposure in a contaminated stream, which was immediately followed by transitory pruritus, ephemeral puffiness of the face, eyelids and upper extremities, shaking chills, fever, diaphoresis, dry cough, generalized body aches, weakness, anorexia, abdominal cramps and watery diarrhea developed and there was a 10 pound weight loss. The persistence of symptoms required his hospitalization on the thirty-sixth day after exposure. Physical examination revealed an acutely ill, undernourished boy with a temperature of 101.2°F., a pulse of 100 and respirations of 22 per minute, and blood pressure of 90 mm. Hg systolic and 50 diastolic. He showed generalized lymphadenopathy, scattered wheezes throughout both lung fields, diffuse abdominal tenderness and a moderate enlargement of liver and spleen.

Laboratory findings from the sixth to the ninth weeks after infestation revealed hemoglobin values of 10.2 to 12.8 gm. per cent; leukocyte counts of 18,800 to 23,200 per cu. mm. with 7,200 to 15,400 eosinophils; a serum albumin of 3.5 gm. per cent and globulins of 4.2 gm. per cent; a positive cephalin flocculation test; thymol turbidity tests ranging from 5 to 8.9 units; an abnormal bromsulphalein retention; a rapid sedimentation rate, and S. mansoni ova in the stools and rectal mucosa. Stool and blood cultures were negative for pathogens. Widal, Weil-Felix and brucella agglutinations were negative as well as the urinalysis, blood serology for syphilis, serum bilirubin, prothrombin time, platelet count, bleeding and clotting times and an x-ray of the chest. The stools were negative for amebas, and positive for T. trichiura, A. lumbricoides and N. americanus ova. Rectosigmoidoscopic examination on the thirty-eighth day revealed an injected, edematous mucosa, with small, yellowish elevations and pin-point hemorrhages, as well as tiny superficial ulcerations. The biopsy specimen obtained from a rectal valve was loaded with living S. mansoni ova. By the fifty-third day the mucosa was granular, and only one dead ovum was present in the biopsy material. On the sixty-first day the skin test with cercariae antigen was positive. The test was negative when the adult worm and egg antigens were employed.

Improvement followed supportive therapy. The diarrhea subsided on the forty-second day. The daily high spikes of fever disappeared on the forty-fifth day, although occasional low grade elevations of temperature persisted until the fifty-sixth day. In spite of an improved appetite, there was no weight gain. Fuadin (stibophen) was started on the fifty-eighth day in daily intramuscular doses of 1.5, 3.0 and 5.0 cc. followed by 5.0 cc. on alternate days, for a total of 100 cc. There was a marked painful enlargement of the liver with hypereosinophilia on the third day of treatment. By the fifteenth week he had gained 12 pounds, the liver was 4 cm. under the costal border and the spleen was felt 2 cm. below the left costal margin. The hemoglobin was 10.9 gm. per cent, and the white blood cell count, 21,050 per cu. mm. with 9,200 eosinophils. No S. mansoni ova were present in stools. Twenty-eight weeks after infestation the stools and rectal biopsy specimens were negative for S. mansoni eggs but the liver was palpable 2 cm. below the costal margins. There was no splenomegaly.

COMMENTS

The term acute Manson's schistosomiasis serves to define the acute, febrile, self-limited disease which in some instances follows exposure to the parasite in heavily contaminated waters. Although the clinical picture is variable in severity, the most explosive variant appears to be rather rare, and is frequently overlooked or mistaken for some other acute febrile diarrheal disease. The variability in the duration and severity of this phase may be a factor influencing the infrequency of clinical recognition. The high prevalence, extensive geographic distribution and severity of the disease add importance to its early recognition and prompt treatment.

The full-blown clinical picture of acute schistosomiasis mansoni is seen among those not previously exposed, who are heavily infected during their initial exposure or during several consecutive exposures. It occurs among visitors to endemic areas or in children from these areas who have avoided infection in the early years of life. It is more common among young boys, who escape the vigilance of their parents for the dangerous delight of a swim. Although other mechanisms of infection such as wading through or laundering in infested streams may increase the incidence in the female, heavy infections would seem to be rather rare in them. Perhaps they are naturally resistant, or else fail to seek medical care during the acute phase. Whether this clinical picture is possible among previously infected individuals with a high degree of immunity remains a moot question.[4]

Since the cercariae leave the snail under a phototropic stimulus[33] infection must occur during daylight. The interval of exposure may not be longer than a few seconds. The degree of immunity, massiveness of infection and individual susceptibility may be the most important factors governing the severity of the disease.

Contrary to a rather widespread notion, immediate cutaneous manifestations are infrequent

among those previously unexposed. It must be emphasized that a history of itching and urticaria immediately following contact with contaminated waters in an endemic area is strongly suggestive of reinfection. Only a minority of those exposed for the first time will show the earliest cutaneous manifestations, consequently absence of this phenomenon does not eliminate the possibility of massive infection. There is enough experimental proof to suggest that the earliest cutaneous manifestations are more frequent and intense among those previously sensitized with the antigen of S. mansoni.[29]

Contrary to other reports,[4,5] the incubation period was conspicuously asymptomatic and variable in duration. From other publications it would appear that the prodromata merged with the actual onset of illness.[4] The onset in our series was characterized by its explosiveness, with predominating constitutional symptoms indistinguishable from those of an acute infectious disease.

There is no adequate explanation for the great variability in incidence and degree of severity of the delayed cutaneous manifestations. While some authors[4,5] imply that these are characteristic of the disease, others[6] report urticaria and puffiness of the eyelids and face as relatively mild and infrequent. Perhaps differences in the infecting dose and in the extent of the hypersensitivity state are the main factors governing these phenomena. Although purpuric manifestations are considered infrequent, they were noted in a previously reported case[5] and in one of this series. Urticaria, puffiness of the eyelids and face, and purpuric manifestations are considered allergic in origin; these phenomena are best explained by an increased capillary fragility.

Fever, the main feature of the acute phase, was self-limited, variable in duration (seventeen to seventy-four days), and always subsided by lysis. It must be emphasized that in those with lighter infections the low-grade fever and mild toxemia of shorter duration may serve to obscure the diagnosis. After defervescence, either spontaneously or following treatment, progressive improvement ensues. This probably marks initiation of the relatively latent state characterized by continuous oviposition and progressive fibrosis.

The discrete, generalized enlargement of lymph nodes observed in all our cases has not been emphasized in the past. We have some evidence to indicate that the lymphadenopathy is due to a non-specific hyperplasia of the lymph nodes. The frequent splenomegaly represents a challenge to the examiner, being easily missed, because of the softness of the spleen, even when the organ extends 3 cm. below the costal margin.

Prior to the tenth week after exposure, the mucosa of the sigmoid and rectum presents the characteristic picture of extensive edema, fiery red erythema, numerous petechial hemorrhages, small ulcerations, and pin-point yellowish elevations, presumably schistosomal pseudotubercles. The picture is pathognomonic to the experienced observer, even in the absence of a positive rectal biopsy specimen.

Positive stools were obtained as early as the fifty-eighth day after exposure, but eggs may have been present prior to this date. The failure of rectal biopsy specimen to disclose eggs or pseudotubercles, even when the stools are positive, remains unexplained. Although this procedure is considered dependable in the diagnosis of the chronically infected, and is frequently used to determine the curability of the disease, it would appear that in the early phases of the disease oviposition and extrusion may occur extensively throughout the colonic and rectal mucosa but perhaps be delayed in the rectal valves. A small biopsy specimen from one portion of the rectal mucosa may not be representative of the pathologic alterations occurring in the extensive mucosal surface of the large intestine.

The early appearance of eosinophilic leukocytosis is emphasized because of its importance in the differential diagnosis. It is the first indication of a parasitic infestation, making the diagnosis of typhoid fever and bacillary or amebic dysentery improbable. The relative and actual eosinophilic levels are variable. The marked increases concomitant with further hepatic enlargement following the initiation of therapy with fuadin (stibophen), and their invariability with intensification of the fever, are subjects for speculation.

The progressive fall of hemoglobin values, maximal during the eighth to the fifteenth week after infection, remains unexplained. The probable utilization of red cells as nutrients by adult parasites; an actual blood loss from egg extrusion and rectal and intestinal erosions; decreased intestinal absorption of iron, and the commonly encountered malnutrition may be influential factors in the pathogenesis of the anemia of acute Manson's schistosomiasis.

The pulmonary manifestations appeared at

the time of clinical onset. Their invariable absence during the incubation period (invasive phase with migration of metacercariae through the lungs) is reemphasized. The host-parasite relationship during this phase of the disease remains obscure. However, after the clinical onset, pulmonary findings consistent with bronchospasm and bronchopneumonia, at times represented by radiographic pictures indisguishable from those of infiltrative eosinophilia, were commonly observed during the acute phase.[31]

The usefulness and interpretation of the cutaneous reactions to different schistosome antigens remains unassessed. The cercarial antigen reaction serves as a screening test in the selection of patients for further diagnostic procedures and for confirmation of diagnosis but not for the determination of the severity of the disease. Positive reactions to the cercarial antigen appeared before the sixth week after infestation. The value of the adult worm and egg antigen reactions in the diagnosis and prognosis of the disease requires further investigation.[34]

Acute schistosomiasis must be differentiated from several other acute febrile illnesses. The range of diagnostic possibilities is limited when there is a history of recent contact with contaminated water and by the eosinophilic leukocytosis. Clinically, it closely simulates an enteric fever, and it is only through negative stool and blood cultures, and serologic tests, that the diagnosis of salmonellosis is precluded. Amebiasis and trichinosis are easily excluded by adequate tests. Caution should be exercised in excluding any form of enteric fever in endemic areas to acute schistosomiasis since patients with acute or chronic schistosomiasis are, of course, susceptible to other febrile diseases.

An analysis of the data derived from this study would indicate that even with heavy infestation the earliest phase (period of invasion) of Manson's schistosomiasis may be asymptomatic. It would appear that few if any pathologic alterations result from migration of the metacercariae, probably because of its remoteness from the host's tissues. Although more massive infections may have led to clinical manifestations during the period of invasion, the exclusively intravascular habitat of the metacercariae may be an important factor governing this asymptomatic stage in our cases. This differs from the violent clinical manifestations that may arise from close contact of parasitic larvae with the host's tissues in some instances of early severe ascariasis and uncinariasis.

The variability of the incubation period reported by others, and confirmed by our series, may depend on the infecting dose and the general condition of the host. In instances of heavier infection and poor nutrition the incubation period may be sharply abbreviated. In those of light infection and good nutrition the incubation period may be lengthened, and the condition may be mild or subclinical. The susceptibility of the host is of utmost importance. With immunity, either partial or complete, the symptoms may be either mild or absent. It seems as if only in those who lack immunity, who are heavily infected, and who suffer from malnutrition or intercurrent diseases would the explosive effects of acute schistosomiasis mansoni develop.

Whether allergic phenomena occur during the earliest phases of Manson's schistosomiasis (period of invasion) in man remains a moot question. There is evidence to suggest that eosinophilia is rather infrequent during the stage of invasion. It seems as if the hypersensitivity state is ushered in by maturation and oviposition. This is substantiated by the invariable presence of eggs in the stools at onset, and favors the close contact of the adult parasite and/or their eggs with the host's tissue as the precipitating and governing factor in the production of symptoms, including the delayed allergic manifestations of puffiness of the eyelids and face, urticaria and purpuric manifestations.

The progression of the disease may be determined by the extent and severity of the hypersensitivity state. It may be perpetuated by the effects of the products of live adult parasites and/or their ova, or from those of their dissolution, with the additional deleterious mechanical effects of ova and/or parasites mainly in the liver, intestines and lungs.

The pathogenesis of the invariably present generalized lymphadenopathy is a subject for speculation. Whether it supervenes from a violent immunologic response, localized reaction of trapped metacercariae migrating through the lymphatics or a combination of both factors, remains undetermined. The rather frequent hyperglobulinemia with increased gamma globulin favors an immunologic reaction. The alteration in serum proteins cannot be entirely attributed to the self-limited, at times rather transitory hepatic dysfunction.

The severity of the clinical manifestations in

acute Manson's schistosomiasis depends in part upon organ hypersensitivity. The symptoms are mainly referred to the gastrointestinal tract, liver and lungs. The degree of severity of the allergic state may be measured by the eosinophilic response. The persistence of intense eosinophilia appears to represent a constant, unrelenting allergic response with the adult parasite and ova as the source of allergens. The chills, fever, diaphoresis and headache are manifestations of toxemia comparable to those of malaria or any other severe infectious disease. The early explosive diarrhea is part of the hypersensitivity reaction with the probable additional mechanical component of egg extrusion through the intestinal mucosa. It must be reemphasized that in chronic cases patients with numerous ova in the stools and rectal mucosa may suffer from constipation, rendering the mechanical component an improbable cause of the diarrhea. The accompanying nausea and vomiting militate in favor of an allergic causation of the gastrointestinal symptoms. It would appear that the early diarrhea results mainly from extensive congestion and edema of the intestinal mucosa with an accompanying disturbance in absorption of fluids.

That the intestinal response to acute Manson's schistosomiasis is an allergic reaction is further substantiated by the enanthematous appearance of the rectal mucosa and, although the local action of ova is contributory, this also is allergic in nature. The local reaction to eggs and the ulcerations of the rectal mucosa are contributory in producing and aggravating the tenesmus. The rectal ulcerations and bloody stools are not benefited by specific antibiotics, rendering a bacterial etiology as improbable.

The frequent splenomegaly appears as a counterpart of the generalized lymphadenopathy. It may be associated with an immunologic response comparable to the acute splenic tumor of an acute infectious disease (typhoid fever). With the development of immunity, the lymph nodes and spleen appear rapidly to return to normal. Both phenomena (splenomegaly and lymphadenopathy) are probably related to the explosive hypersensitivity state that governs the clinical manifestations of the disease.

The invariable hepatomegaly seems to result from the local effect of the adult parasite and their eggs, as well as from the general effects of the allergic state. The exaggerated hepatomegaly with concomitant increases in the eosinophils following the initiation of therapy with fuadin (stibophen) suggest either a direct effect of the drug on the parasites with overproduction of allergens; migration of the injured parasites into the hepatic venules with partial obstruction of the hepatic venous circulation; death and dissolution of the adult parasites and eggs with an increased allergic reaction; the hepatotoxic effect of the drug; or a combination of all these factors.

Permanent hepatic pathologic alterations apparently persist after an acute infection with S. mansoni. The multiple pseudotubercles with eosinophilic infiltration encountered in biopsy specimens of the liver 1.5 years after infestation favor long-lasting allergic and mechanical influences. Whether other factors favoring the development of cirrhosis of the liver affect the already damaged organ cannot now be stated. The persistent damage to the liver may be too mild to be properly evaluated by available tests. There is clinical evidence to show that the hepatic alterations need not interfere with normal development if there is a good diet and if early, adequate suppressive treatment is given.

The earliest pulmonary manifestations may result from migration of the metacercariae through the lungs. These appear to be of little clinical consequence. During the stage of maturation and oviposition the pulmonary picture is dominated by a hypersensitivity state. The unproductive cough probably results from extensive egg embolization from the hemorrhoidal plexus and, possibly, from the degenerating maturing metacercariae which were filtered through the liver and trapped in the pulmonary capillaries. The asthmatic manifestations (bronchospasm) are of allergic origin. The unrelenting local effects of egg embolization with overproduction of fibrous connective tissue, intercurrent pulmonary infections and reduction in the pulmonary vascular bed from an accompanying arteriolitis may result in pulmonary hypertension with chronic cor pulmonale.[35–40]

The duration of the acute phase of Manson's schistosomiasis is variable, depending on the general condition of the host (immunity), the severity of the infection and, probably, the promptness of treatment. The picture is dominated by allergic manifestations and spontaneous clinical improvement is achieved only after the development of immunity.

That the liver is always involved in acute Manson's schistosomiasis has been substantiated by the invariable presence of hepatic enlarge-

ment, by the alteration in liver function tests and by the demonstration of the typical pathologic alterations encountered on biopsy.

There is enough experimental proof to support the production of localized hepatic lesions by the adult parasite as evidenced by unisexual infections in animals.[41–43] These consist of heavy eosinophilic infiltration in the periportal spaces around the adult parasite in the intrahepatic portal radicles. The parasite is a source of local irritation or serves as a source of allergen leading to a localized and generalized allergic reaction. Our earliest biopsy material tends to support this view, since eosinophilic infiltration was observed in the absence of worms and eggs in their immediate neighborhood. The areas of hepatic necrosis observed in unisexual experimental infections[41,42] are not common in man.[15]

From very early in the course of the disease, eggs are being lodged in the hepatic venules, and sometimes in sinusoids, provoking the described reactions, which we believe to be of the allergic and foreign body type. The foreign body type of response occurs from the time the egg arrives in the venules, leading to proliferation of the endothelial lining. This is soon followed by eosinophilic infiltration of the portal spaces. It seems probable that the egg is isolated by proliferation of the intrahepatic venules and in later stages the foreign body reaction may appear in the parenchyma of the liver rather than in the venule.[44] Typical pseudotubercles were found in our cases as early as eighty and one hundred fifteen days after exposure. (Fig. 4.)

With the unrelenting progress of the disease, even though treatment may be instituted early, older pseudotubercles with egg shells in their center are observed. At this later time the localized allergic response diminishes in intensity, and the reaction about the egg shell is more like one of organization and healing, although eosinophilic infiltration about pseudotubercles may persist.

Since in some instances periportal fibrosis may occur late in the course of the disease, despite early treatment and normal liver function tests, the question arises as to how heavy infestation with Manson's schistosomiasis must be before hepatic cirrhosis ensues. This point is not easy to elucidate. For one thing, most experimental observations have been limited to relatively short periods of time, in comparison with the evolution of the condition in man. One of us (E. K.) has reanalyzed data previously published and finds that in Puerto Rico, in one thousand consecutive autopsies, hepatic cirrhosis of all types was noted in 7.5 per cent. In that same series, withdrawing the one hundred forty subjects with histopathologic evidence of schistosomiasis, cirrhosis was found in 5 per cent of the uninfected. On the other hand, the incidence of cirrhosis among the one hundred forty subjects with evidence of schistosomiasis was 17.5 per cent. This seems to corroborate what has long been thought about the direct or indirect importance of the Schistosoma mansoni in the production of cirrhosis of the liver.

The transitory increase in hepatic enlargement in association with an exaggerated eosinophilia and accompanied by fever requires some comment. The hepatic enlargement must be the result of both congestion and marked eosinophilic infiltration. In chronic cases hepatic enlargement has been observed in patients treated with tartar emetic[45] and, although biopsy specimens of the liver failed to show typical pseudotubercles, it must be pointed out that hepatic lesions of schistosomiasis mansoni are well dispersed throughout this organ, rendering their inclusion in a single needle sample uncertain especially in milder infections. The eosinophilia is a bone marrow response to the parasitic infestation.

In may be speculated that a poor diet and intercurrent debilitating diseases may serve to intensify the hepatic alterations. These factors may be all-important in proper evaluation of hepatic alterations in endemic areas with low standards of living.[5] In view of these additional contributing factors in the development of hepatic alterations, there are severe limitations to the reliability of the "hepatic index" as presumptive evidence of schistosomiasis in endemic areas.[46]

The alteration in serum proteins mentioned previously may not be wholly attributed to the hepatic changes, even though most of the liver function tests were abnormal, particularly the cephalin cholesterol flocculation, thymol turbidity and bromsulphalein tests. Although we accept other experiences with the far advanced form of the disease,[47] it may be that other factors also are involved. To begin with, the cephalin cholesterol flocculation test may show alterations that cannot be regarded as definitive proof of diffuse damage to the liver but may be the result of altered metabolism of protein.[48] The

hyperglobulinemia, in association with a generalized lymphadenopathy and splenomegaly, may be part of an antigen-antibody response of the cells of the reticuloendothelial system to products of the adult parasites and eggs. Alterations in the bromsulphalein and thymol turbidity tests presumably signify hepatic dysfunction. However, interpretation of the data requires caution when dealing with a febrile disease like acute Manson's schistosomiasis.[49]

The minor quantitative alterations in the serum albumin, the normal prothrombin time in all patients but one, and the correction of the hypoprothrombinemia with intramuscularly injected vitamin K show that at this stage alterations of the liver are still minor. The false positive serologic test for syphilis in one-fourth of the cases points to a disturbance in serum proteins.

It must be reemphasized that since the spleen is seldom affected directly by the adult parasite and/or eggs[14] the commonly encountered splenomegaly probably represents a violent immunologic reaction comparable to the acute splenic tumor in other acute febrile illnesses. It is plausible to suggest that the earliest hepatic and splenic alterations may be due to a severe hypersensitivity state.

The earliest clinical manifestations of importance coincide with the beginning of oviposition and to a considerable extent must represent the reaction of the host to the hundreds or thousands of eggs and their embryos, which are being trapped in venules, mostly in the colon and liver. With progression of the disease the continuous production of allergens may well perpetuate the unremitting effects of the allergic state. The persistence of hypereosinophilia unresponsive to fuadin (stibophen) therapy favors the concept of continuous production of allergens.[50]

The earliest clinical manifestations remain unaffected by early treatment. These symptoms are controlled only by the development of adequate body defenses. However, suppression or eradication of the sources of allergens may help to prevent the perpetuation of the generalized hypersensitivity state, as well as to limit the local effects of allergens or the obstructive alterations from eggs and parasites trapped in capillaries of intestines, liver and lungs. By adequate control of oviposition through the eradication or suppression of the adult parasite, the multiple focal areas of inflammation and necrosis which later may lead to extensive fibrosis may be radically reduced. Unfortunately, there is not available a single antischistosomal agent of invariable efficacy.

Several factors seem worthy of analysis in an attempt to evaluate the effectiveness of fuadin (stibophen) in this series. It is evident that we have dealt with the most violent form of the disease resulting from massive infection in a fairly well nourished, otherwise healthy, young population sample. Whether identical results are obtained in mildly infected patients remains a moot question, since only those with violent clinical manifestations seek early medical attention. There is no adequate explanation for the clinical improvement that generally follows early administration of the drug. Whether it may be radically altered by intercurrent infections or chronic debilitating diseases and malnutrition, even among those with milder infections, remains a moot question.

There are no universally accepted criteria for cure in schistosomiasis. In spite of the 45 to 50 per cent cure rate attributed to intramuscular fuadin (stibophen) in chronic schistosomiasis, there are no generally accepted standard technics of examination to evaluate cure, and adequate follow-up for prolonged periods in large series are frequently lacking.[51] The disappearance of live ova from the stools or rectal mucosa may signify partial eradication of the adult parasite or transitory cessation of oviposition. That this does not indicate effective cure is illustrated by those cases in the present series considered initially to be therapeutic successes. Persistent pathologic alterations and unrelenting eosinophilia are frequently observed in spite of apparent eradication or suppression of oviposition. Focal or diffuse pathologic alterations generally result in extensive pathologic changes with tendency to chronicity, at times leading to extensive proliferations of connective tissue.

In spite of the limited sample studied, follow-up of our cases indicates that, in the heavily infected, the ravages of the disease can only be limited or attenuated by prompt treatment with fuadin (stibophen). Other factors besides the severity of the infection and early treatment may govern the extent of the chronic pathologic alterations. Whether malnutrition, intercurrent infections or chronic debilitating diseases exaggerate the late effects of Manson's schistosomiasis is a subject for speculation. When the load of eggs deposited in the tissues is small, resorption may occur, with only a few scars

remaining. Larger loads may lead to extensive, perhaps permanent and progressive alterations even after early eradication or suppression of oviposition. When this is attended by conditions favoring damage to the liver, cirrhosis may supervene. It appears that mild or moderate, untreated infections may lead to as much hepatic damage as severe, explosive infections treated at the onset. This phenomenon is mainly governed by the extent of oviposition and the general condition of the host.

Extensive studies on the use of several antimony compounds in the treatment of Manson's schistosomiasis has led to numerous and variable claims of efficacy.[23–27] No perfectly adequate antischistosomal agent is available. Clinical proof tends to support fuadin (stibophen) as the least toxic and most effective of all drugs in common use. However, the data derived from the present study would indicate that the drug is not curative in doses of 40 to 100 cc. Repeated treatments with 40 to 60 cc. fail to alter the unrelenting pathologic alterations, and only in a few instances does it suppress oviposition. A single treatment with 75 to 100 cc. of the drug generally suppresses oviposition within thirteen to thirty-three days but fails to alter the clinical course of the disease or shorten the febrile stage. It must be emphasized that suppression of oviposition may persist for as long as five months to one year after an initial course of 60 to 100 cc. by the methods used. Smaller doses (40 to 60 cc.) invariably failed to suppress oviposition during therapy. There is evidence to suggest spontaneous eradication of the adult parasite, or perhaps sterilization of the female after the third year of infection (Cases 1 and 2),* implying acquired immunity as the most important governing factor in the cure of the disease. The development of newer antimonials is awaited with expectation in view of the extensive distribution and severe morbidity of Manson's schistosomiasis.[52]

SUMMARY AND CONCLUSIONS

Acute Manson's schistosomiasis is the self-limited phase of infection which follows the first or several consecutive exposures to the cercariae of S. mansoni. It is characterized by a variable asymptomatic incubation period, infrequent early cutaneous manifestations, an explosive onset with severe constitutional manifestations indistinguishable from those of an acute infectious disease (typhoid fever), and symptoms and signs dominated by gastrointestinal, hepatic and pulmonary dysfunction.

The clinical picture is dominated by a severe hypersensitivity state, the adult parasites and their eggs being the sources of allergens. The severity of the disease depends on the extent of organ hypersensitivity, which is mainly governed by the infecting dose, the general condition of the host and the adequacy of the immunologic response.

The invariably asymptomatic incubation period (period of invasion) observed in our cases, with appearance of hypereosinophilia, pulmonary manifestations and other signs of allergy only after full maturation of the parasite and oviposition, emphasize the poor allergic properties of the metacercariae and their remoteness from the host's tissues.

Some of the liver function tests (cephalin flocculation, thymol turbidity, bromsulphalein) are frequently if not invariably altered in the disease. Hyperglobulinemia with increased gamma fraction is invariably present together with hepatomegaly, splenomegaly and generalized lymphadenopathy. These manifestations are interpreted as indicating a hypersensitivity state as the underlying mechanism of the symptoms.

Serial biopsy specimens of the liver studied from six-seventy-two days to eighteen months after infection demonstrate an early and diffuse eosinophilic infiltration and pseudotubercle formation. Eosinophilic infiltration decreases with development of chronicity. Whether in the pathogenesis of hepatic cirrhosis in Manson's schistosomiasis other factors, such as chronic debilitating diseases and nutritional deficiencies, play a part, remains to be determined.

The data collected from this study would indicate that the clinical alterations attributed to the hypersensitivity state are unaltered by treatment. Early therapy fails to prevent focal lesions in the liver and large intestine but leads to improvement in the severe lassitude and anorexia, with rapid weight increase. It fails to shorten the febrile course or to influence the eosinophilia.

Courses of 40 to 60 cc. of fuadin (stibophen) fail to eradicate the parasite or to suppress oviposition. Repeated treatment with the same doses is of no apparent additional benefit. The administration of initial total doses of 80 to 100

* Considered as successfully cured when twelve and sixteen consecutive stools and rectal biopsy specimens, respectively, failed to show S. mansoni eggs.

cc. may be effective in suppressing oviposition for as long as five months to one year but fails to eradicate the parasites.

It would seem that the main effect of early fuadin (stibophen) therapy, by the methods employed, is the transitory or prolonged suppression of oviposition. Under the circumstances outlined, eradication of the parasite may depend entirely upon the adequacy of the defensive mechanisms of the host.

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Seminar on Diseases of the Pancreas

Tumors Associated with Hypoglycemia—Pancreatic and Extrapancreatic

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PRIOR to 1929 several neoplasms of islet cell origin had been described histologically[1] and in at least one case[2] an association was made between an inoperable malignant tumor of islet origin and clinical hyperinsulinism. It remained, however, for Graham[3] to remove the first functioning islet cell adenoma and effect a cure. Since that time many cases have been documented.[4–7]

CLINICAL ASPECTS

Typically these tumors make their presence known while they are still very small by liberating large quantities of insulin into the bloodstream and the resultant syndrome is indistinguishable from insulin shock. There are some malignant functioning tumors which in their growth can produce pressure effects on the gastrointestinal tract or even significant bleeding into the bowel but these are rare and such effects are more commonly seen in cases of nonfunctioning islet cell carcinomas.

In a thorough review of 398 cases Howard, Moss and Rhoads[6] found an approximately equal sex distribution and that the tumors occurred at any age from the newborn onward, but that the great majority of functioning tumors were found between the ages of thirty-five and sixty years.

Typically a middle-aged person begins to have recurrent attacks of fainting, shock or queer behavior associated with periods of fasting. The attacks characteristically occur before breakfast. Most of these patients discover that they are able to help themselves by eating or drinking sugar in some form. If repeated fasting blood sugar determinations are obtained it will be found that nearly all these patients have levels below 50 mg. per cent. Howard et al.[6] in their large series noted the average minimum fasting blood sugar to be 32 mg. per cent and only six tumors were found in persons in whom the minimal fasting blood sugar was over 50 mg. per cent. Another exception is a recently reported case,[8] in which the minimal blood sugar was 53 mg. per cent but in this instance plasma insulin assay confirmed the diagnosis.

The attacks cannot, however, be correlated with the absolute level of the blood sugar. A person who exhibits symptoms of hypoglycemia on one occasion at a level of 30 mg. per cent may on other occasions have a blood sugar as low as 20 mg. per cent and yet be asymptomatic. This has been found to be true not only in our series of forty-six cases of proved islet cell tumors but also in other clinical conditions, particularly in the course of insulin shock therapy.[9]

It has been suggested that the rate of fall of the blood sugar level is important in determining the type of resulting symptom complex.[10] Slow declines, in general, result in symptoms of central nervous system origin (such as personality changes, queer behavior, anxiety, restlessness, convulsions, hemiplegia and the like) whereas more rapid falls in the blood sugar level evoke a defense mechanism first described by Cannon, McIver and Bliss,[11] who pointed out that insulin-induced hypoglycemia brought about a release of adrenalin. In the group with rapidly falling blood sugar one sees attacks suggesting overactivity of the autonomic nervous system (such as sweating, nausea, vomiting, hot flashes, diarrhea, tachycardia, weakness and the like). In patients whose falling blood sugar levels are somewhere between these extremes various combinations of the two types of symptoms may be found.

The discovery by the patient that sugar can stave off disaster often leads to excessive caloric intake with an enormous gain in weight. This

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tendency to gain weight, if allowed to proceed to an extreme, greatly increases the difficulty and risk of surgery. In the patients with neoplasms in our series this obtained in 72.3 per cent. One woman, our most recent case, gained a kilogram per month over the five-year period during which her disease remained unrecognized. Formerly she weighed 100 pounds, whereas on admission she weighed 251 pounds.

It must be admitted that the blame for much of the weight gained by this group of patients must be borne by the physicians who, because of failure to recognize the true state of affairs, follow the patients for years treating them symptomatically. This is more easily understood when one considers that almost any psychiatric or neurologic difficulty, from transient motor or sensory disorders to psychoses or paralyses, may occur. It is not surprising, therefore, that a high percentage of these patients come to the surgical service by way of other services where their problems have been extensively studied. Suspicion should be aroused in any case by the remarkably consistent temporal pattern of the attacks, which nearly always occur at the same time of day.

It is a matter of record in most series, including our own, that whatever the nature of the symptom complex in a given individual with recurrent spontaneous hypoglycemia the attacks tend to follow the same pattern each time.[9] For instance patients in whom the attacks are characterized by simple fainting rarely have episodes of paralysis. This interesting phenomenon may stem from the fact that the defensive mechanism responds in an identical manner each time in any one individual.

If left untreated these patients may ultimately sustain irreparable damage to the brain or die during an episode of coma. Howard et al.[6] report fifteen deaths from hyperinsulinism in untreated cases.

Differential Diagnosis. Cattell and Warren[7] quote Allen as saying that the difficulties in diagnosis stem from three facts: "(1) The symptoms, when mild, are essentially subjective. (2) When the symptoms are severe they are not specifically pathognomonic. (3) In most cases, the physician must conduct the initial study at a time when the patient is free of symptoms."

Patients with this disorder are best studied in the hospital where careful observation over long periods is possible and laboratory facilities are available so that immediate blood sugar levels can be obtained. Once hypoglycemia has been considered a possibility and repeatedly documented, there is a second diagnostic hurdle. This is to determine the type of spontaneous hypoglycemia. This problem is skillfully and completely discussed in a recent paper by Conn and Seltzer.[10] Unfortunately, in most institutions no practical method for determining insulin levels in the blood is available so the diagnosis must be established by a process of elimination. Table 1 is abbreviated from Conn and Seltzer and serves as a good check-list in running through the possibilities.

TABLE 1*

ETIOLOGIC CLASSIFICATION OF SPONTANEOUS HYPOGLYCEMIA

Organic—recognizable anatomic lesion:
Hyperinsulinism:
Pancreatic islet cell adenoma (s)
Pancreatic islet cell carcinoma
Generalized hypertrophy and hyperplasia of the islets of Langerhans
Hepatic disease
Anterior pituitary hypofunction
Adrenocortical hypofunction
Fibromas and sarcomas
Central nervous system lesions
Functional—no recognized anatomic lesion but explainable on basis of unusual somatic function:
Hyperinsulinism—imbalance of autonomic nervous system
Alimentary hyperinsulinism—rapid intestinal absorption
Hyperinsulinism of infancy
Idiopathic spontaneous hypoglycemia of infancy
Renal glycosuria
Lactation
Severe continuous muscular work
Factitious—surreptitious insulin administration

* From Conn and Seltzer,[10] abridged.

Organic Hypoglycemia. The causes of "organic hypoglycemia" other than hyperinsulinism are usually quite easily documented. When pituitary, hepatic, adrenal or central nervous system diseases reach the point of producing hypoglycemia they are usually advanced and easily detected by the appropriate clinical and laboratory procedures. This may involve the following tests: X-ray examination of the sella turcica, liver function tests and "liver chemistries," the eosinophilic response to both epinephrine injection and ACTH injection,[5] and an electroencephalogram. Since other endocrine disorders may be associated with islet cell tumors, as noted by Crain and Thorn,[5] the physician must be alert

to this possibility. Wermer,[12] of this clinic, has reported an instance of hereditary endocrine adenomatosis variously involving the islets of Langerhans, the pituitary and the parathyroids in five members of one family.

The sarcomas and fibromas referred to in Table 1 are only beginning to command attention in the literature as causes of hypoglycemia and they are poorly understood. Skillern et al.[13] have reported two instances of very large tumors associated with hypoglycemia and reviewed three cases previously published. It is their belief that these tumors are, in fact, atypical functioning islet cell neoplasms of ectopic origin. We have had a similar case which has to date been filed among the unsolved mysteries. The abstract follows.

Case 1. The patient (P. H. Unit History No. 966067) was a forty-five year old woman who was well until six months before admission when she had the first of five attacks of faintness with loss of contact with her environment. The last four attacks occurred before breakfast, and in the last three blood sugar levels had been 30, 35 and 35 mg. per cent. The fifth attack occurred in the hospital and was promptly relieved by intravenous administration of glucose. The patient had been obese most of her life. She weighed 190 pounds at the age of thirteen years.

On physical examination a very large mass was palpable in the lower abdomen which extended up from the pelvis to the level of the umbilicus. On bimanual pelvic examination this was thought to be continuous with the uterus and was considered a fibromyoma. There were no physical stigmata of endocrine dysfunction.

Because of the large size of the pelvic mass it was thought advisable to remove this first before exploring the pancreas for the islet cell adenoma which was postulated. At operation by Dr. John S. Lockwood, on Sept. 2, 1949, an enormous tumor was found which was thought to arise from the left ovary. Dr. D. A. D'Esopo was called to perform a subtotal hysterectomy and bilateral salpingo-oophorectomy for what was considered a carcinoma of the ovary. Tumor tissue was known to have been left behind on the right lateral pelvic wall, where it could not be dissected free. The pancreas was then palpated and no abnormalities were felt. The duodenum, however, was not mobilized for exploration of the head because of the nature of the patient's ovarian tumor and the difficulty of removal.

Examination of the surgical specimen (Surg. Path. No. A-13950) revealed that the tumor incorporated the left adnexal structures. It measured 20 by 16 by 13.5 cm. Over the superior surface it was covered by smooth glistening serosa. This was absent inferiorly where necrotic, friable fragments of tumor tissue were spilling out. On section the tumor was hemorrhagic, necrotic, and consisted of two major masses measuring 5 and 12 cm. in greatest dimension, respectively. In the less hemorrhagic portions the tissue was opaque, white and soft in the large mass, and in the smaller it was more homogeneous, less friable and gray, with a suggestion of translucence and small yellow areas. The lower pole of the mass was attached by remnants of the broad ligament to the lower end of the uterus, right tube and round ligament which disappeared into the tumor. On microscopic examination, the sections failed to disclose any recognizable ovarian tissue in the tumor mass. The tumor cells, where preserved, were thought to resemble epithelial cells, and the first diagnosis was granulosa cell tumor of the ovary. Later interpretation is discussed in the section on Surgical Pathology.

The patient was given postoperative radiotherapy and, to the intense surprise of all concerned with the case, no further attacks of hypoglycemia occurred. She was followed up and frequent blood sugar determinations were made; these were within normal limits. Two years and eight months after operation, while still without symptoms of hypoglycemia, she was found at a follow-up examination to have a recurrence of tumor in the pelvis. This was giving no local symptoms at the time. Her fasting blood sugar was 89 mg. per cent. She left the hospital, to which she had been admitted for study, against advice. She was admitted almost immediately to the Long Island College Hospital where an exploratory operation was done by Dr. Morris Glass, who found nodules of metastatic growth in the omentum and took a biopsy specimen of one of these. Following this procedure she left the hospital; symptoms of hypoglycemia did not develop until a year later, nearly four years after her first operation. She was then readmitted to the Long Island College Hospital where the first fasting blood sugar in her record was 32 mg. per cent; four subsequent determinations were 47, 49 and 62 mg. per cent, respectively. She was re-explored by Dr Harold F. Laroe. His operative note reads in part as follows:

"Upon opening the peritoneum in the right upper quadrant, a large mass about the size of a small grapefruit was encountered, which was extremely fragile and bled profusely. Exposure of this mass revealed that it was retroperitoneal for the most part at the lateral border of the hepatoduodenal ligament and lateral to the second portion of the duodenum. Attempts to control oozing from this mass were unsuccessful, requiring its removal.

"It was removed by sharp dissection, several large vessels being clamped and ligated. This mass had the appearance of brain tissue and was obviously a metastatic tumor. The gastrocolic omentum was then exposed, and in this omentum a second metastatic mass about the size of an orange was found. This was removed and had the same characteristics as the previously described metastatic mass. Attempts to

explore the pelvis were unsuccessful for the most part because of many adhesions present, but a mass could be felt in the cul-de-sac and adherent to the bladder."

No liver metastases were found, and no suspicious lymph nodes. The pancreas was exposed by dividing the gastrocolic omentum, and on palpation no adenoma was found. The posterior surface was also palpated, and the head of the pancreas was examined anteriorly, but no tumor was felt. The metastatic tumor lateral to the duodenum made it impossible to palpate the posterior surface of the pancreas adequately, but the organ was of normal size and appearance. It was not thought advisable to resect because of the metastatic disease.

The patient recovered from the surgical procedure and died fifty-two months after operation for removal of the primary tumor. No postmortem examination was made.

Functional Hypoglycemia. In differentiating the functional causes of hypoglycemia one is rarely confused by failure to recognize excessive renal loss, lactation or excessive muscular work as causative mechanisms. Alimentary or reactive hyperinsulinism, when it occurs in adults, almost always follows a gastric or intestinal resection. In this group the symptoms of hypoglycemia occur from two to four hours after meals and the pattern of the glucose tolerance curve strongly suggests that the stimulus of a meal calls forth more insulin than is required. Restricting carbohydrate intake helps such patients. Ignoring for the moment the infantile types, the remainder of the functional cases constitute a poorly understood, idiopathic group which some regard as representing autonomic imbalance. Certain generalizations can be made about this group: the attacks are usually unrelated to fasting, rarely occur before breakfast; they often occur in relationship to periods of emotional stress; and the fasting blood sugar is not consistently low. Further, the condition is not relentlessly progressive as it is in the organic hypoglycemias. In these respects functional hypoglycemia is in direct contrast to hypoglycemia due to a tumor.

The Glucose Tolerance Test. The glucose tolerance test is usually performed because of academic interest but it is not a reliable diagnostic aid since the flattened curve said to be typical of hyperinsulinism is often not present. Fasting blood sugars when repeatedly found to be extremely low (below 50 mg. per cent) are of far greater diagnostic significance. It is our habit to subject the hospitalized patients to a twelve to twenty-four-hour fast. It is begun at supper time and if no symptoms appear during the night a blood sugar determination is obtained in the morning upon awakening. If it is below 50 mg. per cent the fast is terminated; if it is not, the fasting is carried through the balance of the twenty-four hour period. If this procedure fails to provoke a significant fall the addition of muscular work (such as stair-climbing) may be imposed to re-enforce the effect and bring to light an otherwise latent case.

The Selection of Cases for Operation. In 1938 Whipple[14] described the clinical triad which has since borne his name. It is as follows: "(1) Attacks of nervous or gastrointestinal disturbances, coming on in the fasting state associated with—(2) Hypoglycemia with readings below 50 mg. per cent. (3) Relief of symptoms by the ingestion of glucose."

If these criteria are rigidly observed in the selection of cases for operation functional islet cell tumors will be found in the great majority.

We rely to a great extent on these criteria, the "Whipple Triad," but at the same time it must be admitted that in spite of the high degree of security offered by the triad it serves only to establish beyond all doubt the presence of a profound recurring hypoglycemia. It is no more specific than that (see Cases I and II). It is therefore essential that the physician consider the differential diagnosis of hypoglycemia as outlined earlier in this discussion before proceeding with an operation. These efforts will help to screen out a few more cases in which surgery might not be needed.

SURGICAL PATHOLOGY

In the Columbia-Presbyterian Medical Center fifty-six patients with hypoglycemia have been explored for islet cell tumor. In nine patients no tumor was found. Five of these were children. Of the four adults in whom no tumor was found only one had the typical Whipple triad and this patient had no relief of symptoms after partial pancreatectomy. He committed suicide two months after operation, and it seems fair to assume he had an undiscovered adenoma. The other three without the typical triad had Van Gierke's disease (one patient) and hypoglycemia factitia (two patients). (Table II.)

Four patients had malignant tumors proved by the presence of metastases. (Table III.) There remain, then, forty-three cases in adults with single or multiple tumors in which operation has

TABLE II

HYPOGLYCEMIA—NO TUMOR FOUND AT OPERATION

(*9 Cases*)

Case No.	Sex	Age	Duration of Symptoms	Lowest Fasting Blood Sugar (mg. %)	Typical Whipple Triad	Nutrition	Date of Operation	Operation	Diagnosis	Follow-up	Autopsy
17.	F	17 yr.	3 mo.	17	No	.....	Jan., 1940	Resection, liver biopsy	Normal pancreas, von Gierke's disease	Died 48 hr. postoperatively	Glycogenosis of liver; hypoplasia of islets
19.	F	7 yr.	1 yr.	42	Yes	Obese	Feb., 1941	Resection	Hypoplasia of islets	No recurrence, 15 yr.; mental retardation	.....
21.	M	50 yr.	5 yr.	36	Yes	Obese	Feb., 1942	Resection	Hyperplasia of islets	Symptoms persisted. Suicide 2 mo. postoperatively	None
32.	F	78 yr.	4 days	24	No	.....	Sept., 1943	Exploration (4 days after hip operation)	Hypoglycemia factitia (not known at time of operation)	Died on table	None
34.	M	27 yr.	33 hr	24	No	.....	May, 1944	Exploration (33 hr. after kidney operation)	Hypoglycemia factitia (not known at time of operation)	Died 15 hr. postoperatively	None
45.	M	14 mo.	10 mo.	24	Yes	Not fat	Nov., 1949	Resection	Hyperplasia of islets	No recurrence, 5 yr.	.....
51.	F	11 mo.	9 mo.	24	No	Not fat	Nov., 1952	Resection	(?) Hyperplasia of islets	No recurrence 2 yr.; being traced	.....
54.	M	2½ mo.	2½ mo.	10	Yes	Not fat	July, 1954	Resection	Hyperplasia of islets	Immediate improvement; mental retardation; lost, 2 mo. postoperatively	.....
55.*	M	26 mo.	26 mo.	13	Yes	Not fat	Mar., 1954	1. Resection body and tail	Hyperplasia of islets	Symptoms persisted	.....
							Apr., 1954	2. Subtotal resection head	Hyperplasia of islets	On ACTH 2½ yr. postoperatively; sometimes spills sugar	.....

* Case II abstract.

TABLE III

HYPOGLYCEMIA—MALIGNANT TUMORS WITH METASTASES FOUND AT OPERATION

(*4 Cases*)

Case No.	Sex	Age (yr.)	Duration of Symptoms	Lowest Fasting Blood Sugar (mg. %)	Nutrition	Date of Operation	Operation	Tumor		Diagnosis	Follow-up	Autopsy
								Site	Size (cm.)			
18.	M	53	3 mo.	30	Not fat	Apr., 1940	Exploratory, biopsy	Ectopic, above head with extension to liver	(Biopsy)	Islet cell carcinoma of aberrant pancreas with extension to liver	Persisting symptoms; died 4 mo. postoperatively	Outside hospital,* metastases (or primary ?) in liver, also in regional lymph nodes, adrenal, kidneys, lungs and subcutaneous tissue
39.	M	57	4 yr.	37	Obese	Aug., 1946	Resection	Tail	2.5	Islet cell carcinoma of pancreas with metastasis to regional lymph node	Died 5 days postoperatively; renal shutdown	None
44.	F	45	6 mo.	30	Fat	Sept., 1949	Panhysterectomy, excision of tumor	(?) Ovary	20.0	Carcinoma, type (?) of (?) ovary with metastases and hypoglycemia	Recurrence symptoms 4 yr.; two reoperations elsewhere; no tumor pancreas; metastases peritoneum; died 52 mo. post hysterectomy.	None
46.	M	19	4 mo.	24	Thin	June, 1950	Exploratory, biopsy (treated with alloxan)	Pancreas	(Biopsy)	Islet cell carcinoma of pancreas with metastasis to lymph nodes and liver	Died 14 days postoperatively	Islet cell cancer of pancreas, metastases to lymph nodes, liver and duodenum; volvulus and obstruction of jejunum; portal vein obstruction; biliary cirrhosis

* Ballinger, J. Hypoglycemia from metastasizing insular carcinoma of aberrant pancreatic tissue in the liver. *Arch. Path.*, 32, p. 277, 1941.

given complete relief of symptoms for long periods of time.

The first thirty-nine of these cases were Dr. Whipple's personal series.[4,15–17] There is a possible ten-year follow-up on forty cases of the total of fifty-six. If we exclude the postoperative deaths, the carcinomas, the nine cases in which no tumor was found—at the time of reporting or subsequently—and the deaths from intercurrent disease, there are twenty-three adult patients who have survived from ten to twenty-two years. Nine of these have lived longer than fifteen years. It is from this long follow-up that we now have a basis on which to evaluate the significance of certain gross and microscopic findings which puzzled us greatly in the earlier days of our study.

Our findings are, in general, in accord with those reported in recent reviews, notably the painstaking and thorough review by Howard.[6] Howard's report of 398 islet cell tumors includes tumors found at autopsy and also 134 non-functional tumors. His total functional islet cell tumors found at operation is 211, including seventeen of those in our series. Clinically, the functional islet cell tumors were benign in about 90 per cent of the cases, and were multiple —including those cases with adenomatosis—in about 10 per cent. The figures are given roughly because longer follow-up has changed the original interpretation since the publication of Howard's review.

In Howard's series for all types of cases—benign, malignant, functional, non-functional, found at operation or found at autopsy—the sexes, in the 235 cases where sex was stated, proved to be 120 females to 115 males. In our forty-seven functional tumors, however, there were thirty-three females to only fourteen males. Our age incidence was also different, being somewhat lower. The majority of the patients with functional tumors in his review were thirty-five to sixty years of age, whereas in ours thirty-five of forty-seven were under fifty and eleven were under thirty years of age.

The location of the benign functional tumors was essentially similar in both series. These tumors can occur anywhere in the gland, and the number in the head is quite high, a matter of concern to the surgeon. Functional tumors have been overlooked at operation in all parts of the gland and, according to Howard, ectopic functional tumors were also overlooked three times. He states, "There is no evidence that the benign adenoma is a premalignant lesion." Our evidence agrees with this. He also says, "Neither the benign adenoma nor the suspiciously malignant tumor has been shown to invade beyond its capsule nor to recur after local excision." We now have to make an exception to the latter statement. One of our questionably malignant tumors was found as a recurrence nine years after the first operation, symptoms having recurred a year before the second exploration. At this operation no metastases were found, so that the recurrence is not a proved malignancy; further study of this case, unfortunately, is not possible because the patient was one of our postoperative deaths, and no postmortem examination was done.

TABLE IV
FUNCTIONAL TUMORS REMOVED AT OPERATION

	Authors' Series		Howard's Series	
	No. of Tumors	Per cent	No. of Tumors	Per cent
Benign	30*	63.8	154	73.0
Questionably malignant	13*	27.7	40	18.9
Proved malignant	4	8.5	17	8.1
	47	100%	211	100%

* Of the forty-three benign or questionably malignant tumors eight, or 18.6 per cent, had multiple tumors of which five were eventually proved to be adenomatosis.

Localized Adenomas. These we have classified,[15] on histologic grounds, as (1) benign and (2) questionably malignant. (Tables v and vi.) We watched our early cases with great apprehension, and were surprised that those tumors which had blood vessel invasion, capsule invasion, cellular variation and frequent mitoses did not seem to give further trouble. These criteria, however, which are reliable in neoplasms generally are not always valid in endocrine tumors, as for instance in thyroid and parathyroid tumors, and this seems to hold also for the endocrine tumors of the pancreas. However, no one could be sure of this until recently, when a sufficiently long follow-up was at hand. So benign has been the clinical course of almost all the suspiciously

TABLE V

HYPOGLYCEMIA—TUMORS FOUND AT OPERATION CONSIDERED BENIGN
(*30 Cases*)

Case No.*	Sex	Age (yr.)	Duration of Symptoms	Lowest Fasting Blood Sugar (mg. %)	Nutrition	Date of Operation	Operation	Tumor		Diagnosis	Follow-up	Autopsy
								Site	Size (cm.)			
1.	F	33	1 yr.	30	Not fat	Jan., 1934	Excision	Junction body and tail	1.5	Benign adenoma	No recurrence, 22 yr.; gastrointestinal bleeding (? duodenum) 19 yr.	.....
2.	F	48	9 mo.	34	Obese	June, 1934	Excision	Neck	1.3	Benign adenoma	No recurrence, 22 yr.	.....
3.	M	28	3 yr.	38	Obese	Aug., 1934	Excision	Neck	1.4	Benign adenoma, multiple	No recurrence of hypoglycemia; died elsewhere 18 mo. "duodenal hemorrhage"	None
						Sept., 1934	Excision	Tail	.....			
4.	M	38	12 yr.	30	Not fat	Aug., 1934	Excision	Body	1.5	Benign adenoma, multiple	Recurrence of symptoms 10 yr.; re-explored; subtotal resection; adenomatosis; died 12 days postoperatively	No adenomatous foci in remnant; no metastases; pituitary normal; oxyphile adenomas of parathyroid
								Head	1.0			
5.	F	28	6 yr.	30	Not fat	Dec., 1934	Resection	Body × 2	1.2	Benign adenoma, multiple	No recurrence, 18 yr.; being traced	.....
									0.3			
6.	F	45	4 yr.	28	Fat	Feb., 1935	Excision	Junction body and tail	2.0	Benign adenoma	No recurrence, 21 yr.; mental institution	.....
7.	F	25	6 mo.	40	Fat	Feb., 1936	Resection	No tumor	.....	Normal pancreas	Symptoms persisting	.....
						May, 1940	Excision	Head	1.5	Benign adenoma	No recurrence, 20 yr.	
9.	F	45	1 yr.	26	Not fat	Feb., 1937	Resection	Tail	0.4	Benign adenoma, multiple	Symptoms persisting	.....
						Dec., 1937	Excision	Head	1.5		No recurrence, 19 yr.	
11.	F	22	4 yr.	37	Not fat	Nov., 1937	Resection	No tumor	.....	Normal pancreas	Symptoms persisting	.....
						Feb., 1938	Excision	Head	1.0	Benign adenoma	No recurrence, 17 yr.	.....
13.	F	22	6 mo.	33	Not fat	Aug., 1938	Resection	Body	1.2	Benign adenoma	No recurrence, 17 yr.	.....
14.	F	54	15 mo.	45	Obese	Oct., 1938	Excision	Junction body and tail	1.2	Benign adenoma	Died 56 hr. postoperatively; (?) thyroid storm	Adenomata of thyroid; massive atelectasis
16.	F	50	9 yr.	34	Obese	Oct., 1939	Excision	Junction body and head	1.5	Benign adenoma	No recurrence, 10 yr.; being traced	.....
24.	F	46	21 mo.	46	Obese	June, 1942	Exploratory	No tumor	.....	.....	Symptoms persisting	.....
						May, 1946	Sub-total pancreatectomy	Head	1.0	Benign adenoma	No recurrence, 10 yr., carcinoma of the colon, 1954	.....
25.	F	46	6 yr.	23	Obese	Dec., 1941	Outside hospital	Tail	"Small"	Benign adenoma	Symptoms persisting	.....
						May, 1942	Outside hospital	Body	0.75	Benign adenoma	Symptoms persisting	.....
						July, 1942	Resection	Diffuse	Microscopic	Adenomatosis	No recurrence, 14 yr.	.....
26.	F	22	2½ yr.	34	Obese	Mar., 1943	Excision	Tail	2.0	Benign adenoma	No recurrence, 12 yr.	.....
27.	F	46	1 yr.	35	Not fat	Feb., 1942	Exploratory	No tumor	.....	.....	Symptoms persisting	.....
						Mar., 1943	Excision	Head	1.0	Benign adenoma	No recurrence, 12 yr.; being traced	.....
29.	F	32	2 yr.	37	Fat	Oct., 1943	Resection	Diffuse	Microscopic	Adenomatosis	No recurrence 4 yr.; died, carcinoma rectum	None
30.	F	64	8 yr.	33	Obese	Nov., 1943	Resection tail Excision tumor	Head	2.5	Benign adenoma	Diabetic; died cerebral accident (?) 15 mo. postoperatively	None
35.	F	38	3 yr.	26	Obese	July, 1944	Excision	Neck	1.4	Benign adenoma	No recurrence, 11 yr.	.....

TABLE V (*Continued*)

HYPOGLYCEMIA—TUMORS FOUND AT OPERATION CONSIDERED BENIGN

(*30 Cases*)

Case No.*	Sex	Age (yr.)	Duration of Symptoms	Lowest Fasting Blood Sugar (mg. %)	Nutrition	Date of Operation	Operation	Tumor		Diagnosis	Follow-up	Autopsy
								Site	Size (cm.)			
36.	F	22	2 yr.	29	Obese	Oct., 1943	Exploratory; outside hospital	No tumor	.....	.....	Symptoms persisting	.....
						Dec., 1944	Resection	No tumor	.....	Normal pancreas	Symptoms persisting	.....
						Sept., 1947	Radical pancreatico-duodenectomy; outside hospital	Head	1.5	Benign adenoma	Died 24 hr. postoperatively	Massive pulmonary atelectasis
38.	M	45	2 yr.	41	Obese	Apr., 1945	Resection	Junction body and tail	1.7	Benign adenoma	Died, hemorrhage, 14 days postoperatively	None
41.	F	35	5 yr.	27	Fat	Sept., 1947	Resection	Diffuse	1.6 (largest)	Adenomatosis	No recurrence, 9 yr. (adenoma of pituitary and ? adenoma of parathyroid, clinical)	.....
42.	F	56	4 yr.	30	Obese	July, 1948	Resection	Tail	1.5	Benign adenoma	No recurrence, 4 yr.; died, 4 yr.	Carcinoma of vulva with metastases
43.	F	49	6½ yr.	20	Obese	July, 1948	Resection	Tail	1.0	Benign adenoma	No recurrence, 4½ yr.; suicide, 4½ yr.	None
48.	F	34	18 mo.	32	Fat	May, 1951	Resection	Diffuse	1.2	Adenomatosis	No recurrence, 8 mo.	Peritonitis
						Oct., 1951	Gastrectomy; excision pancreatic cyst	Duodenum	(largest)	Adenoma of aberrant tissue in duodenum	Died, 8 mo.	Adenoma of pituitary, mixed
						Dec., 1951	Repair perforated gastric ulcer	.....	.....	.....	.....	Adenoma of adrenal cortex, hyperplasia of parathyroids
49.	F	37	3 yr.	27	Fat	Oct., 1952	Excision	Tail	1.7	Benign adenoma	No recurrence, 4 yr.	.....
50.	F	64	4 yr.	33	Fat	Dec., 1952	Excision	Tail	1.0	Benign adenoma	No recurrence, 4 yr.	.....
52.	M	56	3 yr.	32	Obese	Oct., 1953	Excision	Tail	2.0	Benign adenoma	No recurrence, 3 yr.	.....
53.	F	32	1½ yr.	34	Fat	Dec., 1952	Exploratory; outside hospital	No tumor	.....	.....	Symptoms persisting	.....
						Dec., 1953	Excision	Head	1.5	Benign adenoma	No recurrence, 2½ yr.	.....
56.	F	35	5 yr.	23	Obese	Sept., 1956	Resection	Tail	2.0	Benign adenoma	Died 22 days postoperatively	Pulmonary emboli, bilateral; multiple venous thromboses, including subclavian vein.

*A Case 28 did not exist as reported in the Annals of 1940. These cases listed as 29, 30 and 31 and also listed in Howard's review are now 28, 29 and 30, respectively. This applies to the cases in this table and in Table VI.

TABLE VI

HYPOGLYCEMIA—TUMORS FOUND AT OPERATION CONSIDERED QUESTIONABLY MALIGNANT

(*13 Cases*)

Case No.	Sex	Age (yr.)	Duration of Symptoms	Lowest Fasting Blood Sugar (mg. %)	Nutrition	Date of Operation	Operation	Tumor		Diagnosis	Follow-up	Autopsy
								Site	Size (cm.)			
8.	M	51	1 yr.	37	Fat	Feb., 1937	Resection tail, excision tumor	Head	1.3	Adenoma malignum	Died fifth day postoperatively; pneumonia	None
10.	M	50	1 yr.	27	Not fat	Jan., 1938	Resection	Tail	1.5	Carcinoma (?)	No recurrence, 14 yr.; died 14 yr.; carcinoma of bladder	None
12.	F	20	1 yr.	26	Obese	Apr., 1938	Excision	Body	1.2	Adenoma malignum	No recurrence, 11 yr.; lost, 11 yr.	.....
15.	M	46	4 yr.	36	Obese	May, 1938	Explored, outside hospital	.....	.....	No tumor	Symptoms persisted	
						June, 1939	Excision	Head	1.5	Adenoma malignum	No recurrence, 10 yr.; being traced	.....
20.	M	49	4 yr.	37	Obese	Oct., 1941	Excision	Head	2.0	Adenoma malignum	No recurrence, 15 yr.	.....
22.	M	49	2½ yr.	45	Obese	Apr., 1942	Excision	Head	1.8	Carcinoma (?)	Recurrent symptoms 8 yr.; reoperated for recurrence 9 yr.; no metastases found, died postoperatively	None
23.	M	20	1½ yr.	33	Not fat	June, 1942	Excision	Head	1.2	Carcinoma (?)	No recurrence, 14 yr.	.....
28.	F	26	6 mo.	41	Not fat	July, 1943	Excision	Junction body and tail	6.0	Adenoma malignum	No recurrence, 13 yr	.....
31.	F	40	3 yr.	40	Obese	Nov., 1943	Excision	Head	1.5	Adenoma malignum	No recurrence, 12 yr.	.....
33.	F	55	3 yr.	40	Obese	May, 1944	Excision	Head	2.5	Adenoma malignum	No recurrence, 6 yr.; being traced	.....
37.	F	55	4 yr.	34	Obese	Jan., 1945	Resection	Junction body and tail	2.5	Adenoma malignum	No recurrence, 5 yr.; died "intestinal hemorrhage" reported by M.D. in Puerto Rico	None
40.	F	47	2 yr.	25	Fat	Sept., 1946	Excision	Head	1.5	Carcinoma (?)	No recurrence, 10 yr.	.....
47.	M	41	2½ mo.	42	Not fat	May, 1951	Excision	Head	1.5	Carcinoma (?)	No recurrence, 5 yr.	.....

malignant tumors that it is now unfashionable to make a diagnosis of malignancy on any criteria other than metastatic spread. It must be remembered, however, that a "pathologist's cancer" may really have the potentiality of malignancy and yet not demonstrate this character because the surgeon has removed it in time, and so "cured" the patient. The term "cure" is, of course, also unfashionable, as it should be, and a ten-year follow-up is the least, in our opinion, on which to consider the patient with either multiple tumors or a tumor of questionable malignancy probably cured. We have re-explored one patient with recurrent symptoms in each of these categories, about ten years after the first procedure which had resulted in an apparent cure, and found at the second operation neoplasms similar to those previously removed.

Gross pathology: Grossly benign islet cell adenomas are often of the same consistency as the surrounding pancreas, and sometimes nearly the same color, perhaps only a little darker yellow before surgical manipulation. The majority, however, have a blue or violet tinge which, because of vascularity and the necessary surgical handling, often becomes a dark reddish purple after removal. In adenomatosis, in addition to the grossly recognizable tumors, the neoplastic and hyperplastic islets appear as violaceous areas against the background of the pale yellow pancreas. In general the benign tumors are small, averaging 1 to 2 cm. in diameter, sharply circumscribed, often really encapsulated. Tumors over 3 cm. in diameter should be regarded with suspicion as being possibly malignant, and hemorrhagic tumors, if found to be so before much surgical handling has bruised the tumor, are also to be viewed with alarm. Very large tumors are usually obviously infiltrating and recognized as such.

Microscopic findings: Microscopically the tumors are made up of cells which, in the great majority of cases, closely resemble the cells of the islets in the surrounding pancreas. The very well differentiated neoplasms suggest gigantic islets. There are several well-defined patterns, only one of which in our experience has any diagnostic significance, but which constitute puzzling findings to the general pathologist not associated with an active pancreatic surgical service. There is a rosette or glandular pattern; a duct pattern in which ducts appear to form the bulk of the neoplasm and the cells look much more like those of the excretory ducts than they do like those of the islets; a pattern in which the viable tumor cells are arranged in broad bands separated by masses of degenerated tissue, sometimes hyaline in appearance, sometimes granular. Some of these patterns are so far from that of the well differentiated gigantic islet that it is hard to accept them as representing islet cell tumors until the patient's clinical course has demonstrated their character because of relief of symptoms by removal of the tumor.

In a further effort to determine the nature of the cells in these tumors, tissue culture studies were undertaken in the fifth case and continued through the thirty-third in our series.[18] The cells grew out as pavement epithelium, with variable degrees of pleomorphism and mitotic activity which at first we thought could possibly be correlated with the benign or malignant nature of the tumor. Twenty-two tumors in all were cultured, and there was growth in all but three. Two of the series were used for grafting into diabetic patients, having been grown in the recipient's serum for two weeks beforehand. These efforts failed to change the insulin requirements of the recipient, nor was there any evidence that the grafts had taken. This was a disappointment.

Treatment with alloxan: In the early days insulin assays were performed on a number of benign tumors as well as on metastases to the liver from the malignant variety. Sometimes both primary tumor and metastases yielded insulin, sometimes only the primary. The functional nature of the malignant tumors was also documented by the administration of alloxan, with subsequent degeneration of portions of the tumor thought to consist chiefly of beta cells.[19] We administered this drug to one patient, a boy of nineteen, in the terminal stages of metastatic functional carcinoma. There was some laboratory evidence that there was a reduction of insulin output after administration of the drug, and at postmortem no changes, such as had been previously described in animals and man as due to alloxan toxicity, were found in other organs. It was impossible, however, in the largely necrotic carcinoma, primary and metastatic, to find changes in the carcinoma cells which might have been due to the specific action of alloxan.

Specificity of islet cells: This specific inhibitory effect on the function of insulin-producing cells, however, is additional support for the belief that the different types of cells found in normal islets serve different endocrine functions. Doubt

has been cast on this, however, by the work of Sergeyeva[20] who reported that by suitable technics she could induce numeric changes in the ratio of alpha and beta cells in the islets of normal animals (cats). It is possible that the morphologic differences between cells in normal islets represent different phases of secretory activity, and that all the islet cells have more than one possible endocrine potentiality.

The first functional islet cell neoplasms surgically removed were studied with great enthusiasm, and with some success in various laboratories by the special granule stains. Most of the benign functional tumors were reported as beta cell tumors, and some of the non-functional adenomas also. Such studies are still widely undertaken. They would be particularly valuable, if uniform results could be obtained, in the differential diagnosis of islet cell tumors and carcinoids, as well as for islet cell, duct-cell and possible acinar-cell tumors of the pancreas. The methods, we regret to report, have not been successful in our hands, although we have had some good results with the technics with normal human islets and with animal tissues. We have, therefore, perhaps indolently, been content to settle for the diagnosis of a tumor of islet cell origin on the basis of general morphology and the clinical result, arguing that the less well differentiated tumors might be atypical in staining reaction because of the neoplastic character of the cells.

Recently Zollinger[21] has suggested that there is a "clinical entity consisting of hypersecretion, hyperacidity, and atypical peptic ulceration associated with non-insulin-producing islet cell tumors of the pancreas." Based on our own series of insulin-producing tumors we have the impression that there is a possible statistically significant higher incidence of gastrointestinal bleeding in patients who have had islet cell adenomas than in patients without such tumors. In the follow-up, excluding the postoperative deaths, of forty patients who had had functional islet cell tumors removed with relief of symptoms of hyperinsulinism, four had gastrointestinal bleeding, which was fatal in three. In two of these the site was known to be the duodenum, and in one the stomach; in the fourth it was not stated. Duodenal ulcer also is part of the clearly hereditary and definite syndrome of multiple tumors of endocrines with adenomatosis of islets.[12] There is probably much still to be learned about the internal secretion of the pancreas.

Hyperplasia in Adults and Children. In the early days of enthusiastic surgery for hypoglycemia, patients without the typical clinical picture were often explored and when no tumor was found a portion of the pancreas was resected and the surgical pathologist was pressed to find something abnormal in the specimen. The diagnosis of "hyperplasia" or "hypertrophy" of islets was frequently made. It is well known that there is great variation in the size and number of islets in different individuals and also in the distribution of islets. Commonly they are found in larger numbers in the tail, and this of course was the portion of the organ removed to reduce the amount of islet-bearing tissue. Early follow-up, on patients with no tumor, often suggested that resection had been effective. In our own study, however, resection of non-tumor bearing tissue has not, in any adult with the typical Whipple triad, led to permanent relief.

We have had no experience with islet cell tumors in infants and children, with or without hypoglycemia, although Dr. Dorothy Andersen[22] states that in more than twenty autopsies, largely on premature infants but also on older infants and occasionally on a young child, hyperplasia of islets, almost worthy of the diagnosis of adenomatosis, has been striking, even though most of the infants were not born of diabetic mothers and only a few had been treated with cortisone. In none of these cases could Dr. Andersen correlate these autopsy findings with hypoglycemia clinically. In thirteen cases of clinical idiopathic hypoglycemia in infants and children, five only have been surgically explored.* These ranged in age from seven years to two and a half months. In none of these was an adenoma found. Reports of cases of functional islet cell tumors in infants and children, however, have appeared in the literature, although pancreatic tumors of any kind are exceedingly rare in these age groups. In the children, who have had more encouraging results of partial pancreatectomy than the adults in our experience, relief has been evident for considerable periods, with diagnoses varying from hyperplasia, normal pancreas and even hypoplasia (this in a girl of seven who has had no evidence of recurrence of symptoms for sixteen years).

Functional Islet Cell Carcinomas. The malignant tumors of islet cells, primary in the pancreas and with characteristic metastases to regional

* Babies Hospital, Columbia-Presbyterian Medical Center. (Table II.)

nodes and liver (and often nowhere else) are for the most part rapidly growing, and have a short clinical course. (It is interesting that the histologically similar non-functional islet cell carcinomas are clinically much more benign than the adenocarcinomas of the pancreas, the patients living for long periods even after metastases to the liver have been documented.) The hypoglycemia of the functional carcinoma patients is often extreme; these patients are usually thin in spite of constant administration of carbohydrates. It is, therefore, the uncontrollable hyperinsulinism rather than the site and complications of the primary tumor and the metastases which leads to the rapid downhill course in these cases.

Gross pathology: There is nothing in the gross appearance of the malignant functional tumors which differentiates them from non-functional islet cell carcinoma or from the much more common adenocarcinoma of the parenchyma. The sites of the primary malignant functional tumors and of the metastases are similar to those of the adenocarcinomas.

Microscopic examination: Some of the carcinomas with metastases have shown great cellular variation and have had a highly malignant character histologically. Others are much better differentiated. Some closely resemble the questionably malignant tumors which have not been proved malignant by follow-up. Some of the tumors with metastases have been so well-differentiated when still localized and small that the finding of metastatic deposit in a lymph node has been a surprise and altered the first impression and diagnosis. It should be said, parenthetically, that the benign non-functional tumors found at necropsy are, in routine laboratory examination, also very similar histologically to those which have given symptoms of hyperinsulinism.

Prognosis by Histology. The one pattern which should give the surgical pathologist pause is a pattern which we presented in our first publication in 1935, calling it a "ribbon pattern." The cells are arranged in what appear to be double cords and are not separated by degenerated material, but by capillaries. We found this in our fourth patient who had two tumors removed at one time, but its significance did not become clear for a good many years. We found it later in our cases of adenomatosis and it could be seen not only in the grossly recognizable neoplasms but in islets which were only slightly hypertrophic microscopically. It has characterized the adenomatosis cases in which there were tumors of other endocrines.[12] To our great interest the fourth patient in the series, the one with the original ribbon pattern, returned after ten years with recurrence of symptoms and on re-exploration was found to have adenomatosis. This pattern, therefore, is considered of diagnostic and prognostic significance.

Malignant Spindle Cell Tumors. The next task of the surgical pathologist is to clarify the cases, of which only five have been published[13] and to which we have been able to add a sixth (Case 1), in which large tumors of undetermined origin have been the apparent cause of attacks of hypoglycemia. These have been called "tumors with spindle cells." So far no insulin assay of any of these tumors has been reported. In our patient the tumor was removed in the full expectation that later it might be necessary to re-explore the pancreas more thoroughly and remove a benign islet cell tumor, even though the pathologist thought the lesion malignant. The patient would therefore be spared from hypoglycemic symptoms in the terminal stages of her malignant disease. It was a complete surprise that the removal of this tumor, thought at operation to have arisen in the ovary, gave relief of symptoms for nearly four years. The recurrent tumor, with metastases, accompanied by recurrence of hypoglycemia, resembled the primary. In spite of prolonged study of the sections from all three surgical procedures, we are baffled by this neoplasm. We are not quite prepared to accept it as a malignant islet cell tumor of aberrant tissue. It could have arisen in the ovary, or possibly in the retroperitoneal tissue, but it is not clearly either epithelial or mesothelial in character. We have heard rumors that similar puzzling cases have been extracted from the files in other laboratories. These may, when published, help us to interpret our own case. If in the future an opportunity offers itself to any reader of this article to suggest an insulin assay of such a fresh tumor, the findings would be of great interest.

THE PROBLEM IN THE OPERATING ROOM

It is our practice to take a patient who is to be explored for an insulin-producing tumor, and who has had nothing to eat during the night, to the operating room at 8 A.M. with an infusion of 10 per cent dextrose in water running intravenously. The infusion should be started at about 6 A.M. to preclude an attack, and should be con-

tinued throughout the operation. We have never observed shock resulting from the sudden release of excessive insulin due to surgical handling of the tumor, although this might be expected because of the analogous problem in endocrine surgery posed by the excision of pheochromocytomas. Although ether is known to raise the blood sugar level and might therefore be considered the anesthetic of choice, its superiority in this respect is relatively unimportant and does not outweigh the other factors which usually govern the choice of an anesthetic. It is therefore customary to pick an agent which is best for a given patient without too much regard for the diagnosis of hyperinsulinism.[22] The glucose infusion should obviate the problem of hypoglycemia during operation.

The problem peculiar to this surgical situation is the matter of finding the lesion. If one considers that the tumor may be only 3 or 4 mm. in diameter, hidden in the substance of a comparatively large gland, which in turn is buried deep within a patient who is not infrequently maximally obese, it will be appreciated that this is truly a surgical needle in a haystack.

The pancreas is not considered to have been adequately palpated until and unless it has been completely mobilized and palpated with fingers behind and in front of it. To do this properly the peritoneum must be divided around the duodenal curve and along the superior or inferior border of the body and tail of the gland. This cannot be over-emphasized. It was not done in some of those patients who had to be explored later because small tumors had been missed on the posterior surface of the pancreas.

It is an error to seize upon an obvious adenoma and hustle out of the abdomen. As has been mentioned, 18.6 per cent of these lesions in our series are multiple, and the operation will fail unless all the adenomas are found and removed. In every case the entire gland is carefully examined and a search is also made for ectopic foci of pancreatic tissue. Ectopic tumors are uncommon, being quoted as in the incidence of 3.6 per cent in Howard's[6] series (one of forty-seven cases in ours). When they occur they are usually in the immediate vicinity of the pancreas.

The excised lesion should be promptly examined by the surgical pathologist—*not* so that he can decide whether it is benign or malignant microscopically (a virtual impossibility in these cases) but to decide whether or not it is an islet tumor (it is possible to mistake a peripancreatic lymph node grossly for an islet adenoma), and whether or not it has been completely excised. If, instead of simple excision, resection has been done, the pancreatic tissue surrounding the dominant tumor should be scrutinized for evidence of adenomatosis.

McMillan[24] has described a case in which he determined the patient's blood sugar at the time of operation in the operating room before and after removing an islet adenoma. In the thirty-minute interval it rose from 131 mg. per cent to 258 mg. per cent. This has no advantage over frozen section in proving that the lesion has been found in the case of a solitary tumor, but it might prove to be valuable in detecting the presence of undiscovered multiple tumors. We plan to make these determinations in the future in an effort to determine the reliability of the method. It may well save the patient further exploratory procedures, with all the technical difficulties they present.

No Lesion Found. If the work-up leading to the decision to operate has been carefully and completely performed and the diagnostic criteria fulfilled the surgeon will feel more secure in deciding to resect most of the gland when, after careful exploration, no adenoma is found. (See section on Hyperplasia.) In adults it is our practice to remove all the pancreas to the left of the superior mesenteric vessels. This necessitates an associated splenectomy. The resected specimen is given to an experienced surgical pathologist who carefully sections the gland at intervals of a few millimeters. If a tumor is found nothing further is done. If no tumor is found most of the head of the pancreas is removed, leaving enough gland to make duodenal and bile duct resection unnecessary. This is similarly sectioned and examined by the pathologist. If no lesion is found we now believe, on the basis of our own experience, that it is warranted to complete the radical pancreaticoduodenectomy at this first procedure. The remainder of the gland is removed using the usual technics for duodenal and bile duct resections.

In one of the Presbyterian Hospital cases, the patient was subjected to a resection which removed the tail, body and much of the head of the pancreas. This procedure failed to relieve the patient's symptoms and her hypoglycemic attacks became more frequent and more severe so that in order to abort them she increased her carbohydrate intake greatly. At a third operation at another hospital a pancreaticoduodenec-

tomy was performed, removing the remnant of the head of the pancreas. Neither the surgeon nor the pathologist (nor an interested by-stander, Dr. Whipple himself) could palpate the tumor in the resected specimen. On section, however, a 1.5 cm. benign adenoma was found next to the ampulla of Vater. The patient by the time of this operation, five years after the onset of illness, was enormously obese, although only twenty-five years old. The procedure was very difficult because of this and the scar tissue. She died within twenty-four hours with extensive pulmonary atelectasis.

This case illustrates the difficulties and the increased risk of reoperation. We believe now, on the basis of this and other cases, that radical operation at the first procedure is justified, and henceforth plan to practice it regularly in adults. One can easily understand that the surgeon faced with procedures of this magnitude must have complete confidence that the anteoperative work-up leaves nothing to be desired. Fortunately, many adenomas are relatively easy to find and so situated as to permit their being "shelled out." The necessity to resect blindly, however, has occurred in fifteen of fifty-two patients (excluding the four operated upon *in extremis* for diagnosis) or 28.8 per cent.

Our experience, as has been stated, does not give us the right to stop with a resection of the tail and body of the gland. With the one exception of the patient with persisting symptoms who committed suicide before a further operation could be performed, we have had to re-explore every adult patient in whom no tumor was found originally and in whom no other cause for hypoglycemia was evident. There has been temporary alleviation of symptoms in some cases of partial pancreatectomy when no tumor was found but, sooner or later, these, in our experience, have come to re-exploration even though the pathologist sometimes had made a comforting diagnosis of hyperplasia on the original tissue resected.

Howard et al.[6] found forty-six patients in the literature who were subjected to "blind resections" and in whom no tumor was ever found. Thirty-six had been previously reported by David and Campbell,[25] including two of the authors' own. The reported follow-up period in these cases was often short. In our own cases (some included in Howard's table) the passage of years has thrown light on a number of the failures, and darkened some of the good results. Improvement in the selection of cases for operation will, we believe, yield a much higher number of occult tumors found in the "blind resections."

The Pediatric Problem. Insulin-producing tumors do occur in the first decade of life[6,26] but they are rare. As has been mentioned, the files of the Babies Hospital contain no instance of a true islet cell tumor.[21] On the other hand, spontaneous hypoglycemias of other types, such as McQuarrie's[27] idiopathic spontaneous hypoglycemia of childhood, are not too infrequent. This poses a difficult problem to the pediatricians and surgeons faced with a patient exhibiting a syndrome which in an adult would necessitate exploration.

In the past this dilemma was solved by exploring and doing partial pancreatectomies (tail, or tail and body) on empiric grounds (see Case II). In the light of the findings of McQuarrie it is our intent to approach youngsters with serious degrees of spontaneous unexplained hypoglycemia in a different manner. They will be given a trial of ACTH according to the scheme outlined by McQuarrie and if major improvement does not occur they will then be explored.

If no adenoma is found the body and tail of the pancreas will be resected. Even if the pathologist finds hyperplasia of the islets no further resection will be done. This plan of operative attack differs from our more aggressive approach in adults simply because our experience indicates that, in children, resection of part of a pancreas showing either islet hyperplasia or no abnormality at all may be associated with relief of hypoglycemia. Gross[28] in his published series reports a similar experience with such resections. If this plan proves to be unsuccessful in any individual further resection could be performed. The complexities of this problem are well illustrated by Case II.

CASE II. The patient (P. H. Unit History No. 155742) was first admitted to the Babies Hospital on December 1, 1953 at which time he was twenty-four months old. The mother reported the child had been having convulsions since birth. By the time he was three months old they were occurring on the average of two times a day. One of these attacks usually occurred before breakfast. Typically his eyes became crossed, he became unresponsive, fell, and began to have clonic and tonic movements of all extremities. The attacks varied from one to thirty minutes and ceased spontaneously. An hour of lethargy would follow. There was no known birth trauma. No evidence of endocrine dysfunction was elicited. Physical

and neurologic examinations were negative. Psychometric examination was of questionable reliability but the patient seemed to be below normal in all areas tested. The electroencephalogram was normal. The Mazzini test was negative. The hemoglobin was 11.3 gm. per cent, the white blood cell count and differential were normal. The urine was normal. The Mantoux test was negative. The serum sodium, calcium, CO_2 and chloride levels were all normal. The cephalin flocculation test was negative. Spinal fluid showed a protein of 28 mg. per cent and sugar of 13 mg. per cent. Many fasting blood sugar determinations were made and were found to range from 7 to 67 mg. per cent, most of the readings being in the 20 to 35 mg. per cent range.

He was then given ACTH, 20 mg. every six hours intramuscularly, for one week (the child's weight was 12,050 gm.). This had no apparent effect on the daily blood sugar levels. The astonishing thing to all who observed the patient was that he never had an attack in the hospital even when his blood sugar was down to a level of 7 mg. per cent. On March 11, 1954, 60 per cent of the pancreas (body and tail), together with the spleen, was removed. The gland was grossly normal. A pathologic report of diffuse hyperplasia of the islet of Langerhans was made. A postoperative needle biopsy of the liver was interpreted as normal.

The child made a normal recoery from the operation but was not improved in respect to his blood sugar levels. Accordingly, on April 19, 1954 he was operated upon again. The remaining pancreas was grossly normal and all of it except for a little shell over the common bile duct was removed. It was judged that less than 10 per cent of the gland remained. The pathology report on this fragment was the same as on the previous one. Again his recovery was rapid and complete. A small abscess developed postoperatively but it was without consequence. The blood sugar levels remained low.

He returned home and soon began to have convulsions. He was readmitted on July 25, 1954 and a prolonged period of experimenting with various dosages of ACTH, cortisone and meticortin was started. It became apparent that cortisone, in doses small enough to avoid undesirable side effects, produced only a temporary elevation of the blood sugar, of short duration. Intramuscular ACTH produced a prolonged effect and he was regulated on a daily dose of 75 mg. which kept the blood sugar above 50 mg. per cent. He lived at home most of the time although he had frequent admissions for study. In December 1955 an infection developed and while in the hospital because of the finding of a white blood cell count of 20,800 he had a bone marrow study which showed normal marrow except for the presence of scattered large pale cells which seemed to be actively phagocytic. These resembled cells seen in Niemann-Pick's disease.

He was in again in August 1956 with tonsillitis from which he made a rapid recovery. At present he is being given daily intramuscular injections of 7.5 mg. of ACTHAR gel at home. His blood sugar is usually over 50 mg. per cent and he not infrequently spills sugar in his urine.

POSTOPERATIVE PROBLEMS

The incidence of the usual complications of major abdominal surgery is made greater by the extraordinary obesity in hypoglycemic patients who come to surgery late in the course of their disease. This applies not only to wound complications but also to atelectasis and thromboembolism. There are, however, certain complications peculiar to this clinical problem.

Diabetes. Most patients have a transient diabetes for from one to ten days after operation. In our experience this has always cleared up. There are, of course, reports[29] of permanent diabetes following removal of tumors by total pancreatectomy. This diabetic phase should be anticipated and treated appropriately in the immediate postoperative period. We have had two or three patients who became acidotic on the night of operation but we have not seen the onset of such diabetes delayed beyond the first twenty-four hours after operation.

Fistulas. Any incision into the pancreas may result in a fistula and our patients' wounds are all drained with this possibility in mind. It is unusual to find a significant persistent volume of drainage unless a major duct in the head of the gland has been lacerated. The treatment of these persisting fistulas is beyond the scope of this paper. Pseudocysts, fat necrosis and abscesses also may occur but are relatively rare.

Hyperthermia. Deaths due to unexplained postoperative hyperthermia following excision of functional islet cell adenomas have been reported in the literature.[10] In our series of forty-four cases in which localized tumors were resected only one patient might fall into this category. This patient may have had a thyroid storm. With this one possible exception the postoperative fevers were all explainable.

The Role of ACTH. Conn[10] recommends that ACTH be used to prepare these patients for surgery on the ground that it is desirable to "elevate the blood sugar by inducing resistance to the activity of excessive quantities of insulin" and possibly also to prevent the occurrence of "idiopathic hyperthermia." In our experience preoperative control of the hospitalized patient's blood sugar by ordinary methods has presented no difficulties. As already mentioned, good for-

tune has, to date, spared us the experience of having to cope with idiopathic hyperthermia.

We are, however, very much interested in the possibility of controlling poor-risk patients for variable periods preoperatively, and for this reason plan to use steroids in preparing all our patients in the future in order to evaluate the usefulness of this measure. One wonders whether or not it would be possible to maintain a very obese patient on steroid therapy long enough to effect a significant weight reduction; if so, it would be a major contribution to the safety of operation. Patients who are poor risks for reasons other than obesity might also profit by a longer preparatory period before operation.

In the one case in which we have already used steroids there was no significant change in the patient's daily fasting blood sugar. This may be because of the dose, which was only 80 mg. per day over a period of twelve days, after which, at operation, a typical benign adenoma was removed.

Operative Mortality. Exclusive of four patients operated upon *in extremis* for diagnosis only, there have been fifty-nine operations for hypoglycemia in fifty-two patients at the Columbia-Presbyterian Medical Center. Five patients had had previous explorations in outside hospitals and two were re-explored later in outside hospitals. There were therefore sixty-six operations upon fifty-two patients in the hypoglycemic state. Following up our own fifty-nine operations there were seven postoperative deaths, or an 11.9 per cent mortality. Two of the seven were re-explorations. (In a third case, the patient died at re-exploration at another hospital.) This is a strong argument for early diagnosis—so that the perils attendant upon obesity are minimized—and also for adequate resection at the first procedure.

SUMMARY

In summary we would like to emphasize the following points.

1. The diagnostic work-up of the hypoglycemic patient must be carried out very carefully in order to justify the frequent necessity for "blind resection."

2. The microscopic entity, hyperplasia of the islets of Langerhans, has never in our adult series been responsible for clinically significant hypoglycemia.

3. In infants and children the correlation between clinically significant hypoglycemia and abnormal islets of Langerhans is not the same as in adults. The therapeutic approach is different.

4. A five-year follow-up is not long enough to rule out the possibility of recurrent hypoglycemia due to recurrent or persistent neoplasm.

5. Another case of a large extrapancreatic tumor associated with hypoglycemia is added to the five reported in the literature. We cannot classify it.

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Clinico-pathologic Conference

Chills, Fever, Dyspnea and Cyanosis

STENOGRAPHIC reports, edited by Amoz I. Chernoff, M.D. and W. Stanley Hartroft, M.D. of weekly clinico-pathologic conferences held in the Barnes and Wohl Hospitals, are published in each issue of the Journal. These conferences are participated in jointly by members of the Departments of Internal Medicine and Pathology of the Washington University School of Medicine and by Junior and Senior medical students.

THE patient (No. 91404), a Negro woman elevator operator, forty-four years of age, was admitted to the medical service of Barnes Hospital on December 31, 1955 with the chief complaint of chills and fever of three days' duration.

The patient was in good health until two weeks prior to admission when she noted fatigue and aching in both shoulders which she ascribed to unusually hard work. Three days before admission malaise, headache, myalgia and anorexia developed; she had a shaking chill followed by a feverish sensation. These symptoms persisted with daily or twice daily episodes of chills and fever until the day of admission. On December 29, 1955 she noted mild exertional dyspnea and a cough productive of small amounts of tenacious mucoid sputum. At no time did she note hemoptysis or chest pain. The next day, while washing her hands, she suddenly lost consciousness, fell and struck the back of her head. She recovered promptly and noted no sequelae. Because of the persistence of her systemic symptoms she came to the emergency room and was admitted to Barnes Hospital. The patient denied any contact with sick animals or birds. She had not attended any sick patients and had taken no medications.

About 1925, the patient had had an appendectomy and an operation for "pus tubes" at another hospital. In 1941, she was admitted for four days to Barnes Hospital because of acute urticaria. Her symptoms subsided spontaneously and did not recur. About two weeks prior to her final admission the patient noted swelling of her face, feet and hands, for which she was advised to limit the salt in her diet. After avoiding table salt for several days she noted the subsidence of swelling and a weight loss of 2 pounds.

The family history was non-contributory except for the fact that her mother died at the age of forty-eight of "pneumonia."

Physical examination at the time of admission revealed the temperature to be 39.2°C., pulse 92, respirations 26 and blood pressure 115/65. The patient was obese and appeared mildly ill but was not cyanotic, dyspneic or orthopneic. The skin was unremarkable and there was no lymph node enlargement. Eyes, ears and nose were not abnormal. The posterior pharynx was moderately injected without any exudate. The neck was supple and the trachea was not deviated. The chest was symmetrical. At both bases the percussion note was impaired but the breath sounds were normal. Fine, medium, moist rales were heard over both lung fields posteriorly and in the axillas. The heart was not enlarged. The rhythm was regular. A_2 was greater than P_2 and a grade II systolic murmur was heard over the entire precordium, loudest at the apex. The abdomen was soft, non-tender and without palpable masses. Pelvic and rectal examination revealed no abnormalities. The extremities were unremarkable and the neurologic examination was within normal limits.

Laboratory data were as follows: hemoglobin 11.3 gm. per cent and white blood cell count 11,900 per cu. mm. Differential: eosinophils 2, band forms 6, segmented neutrophils 67, lymphocytes 22 and monocytes 3. The red blood cells and platelets appeared normal. Urine: specific gravity 1.025, pH 5.5, protein negative, sugar negative, microscopic negative. The stool was brown and guaiac negative. The cardiolipin test was negative. Culture of the blood revealed no growth. Culture of the throat revealed heavy growth of alpha-hemolytic streptococci and moderate growth of neisseria. Culture of the nose revealed a few colonies of staphylococcus albus. Roentgenograms of the chest showed bilateral generalized pneumonia. (Figs. 1 and 2.)

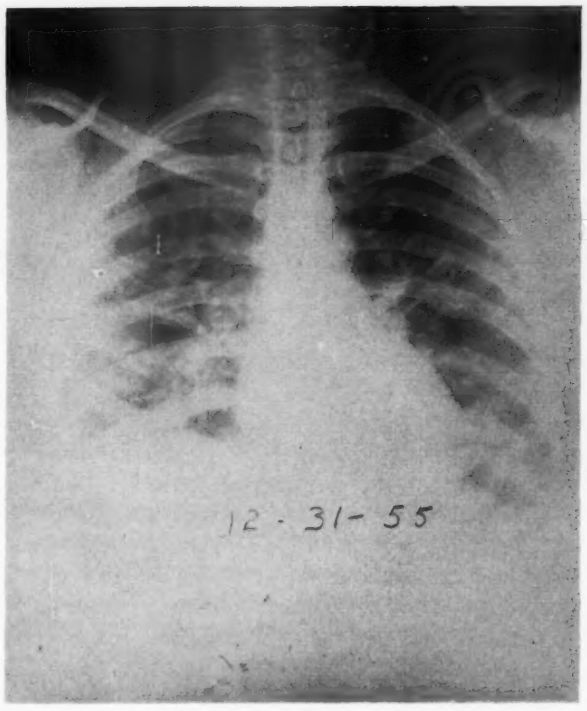

FIG. 1. Chest roentgenogram, posteroanterior view, December 31, 1955.

FIG. 2. Left lateral projection of chest, December 31, 1955.

Electrocardiogram showed a borderline record. Non-protein nitrogen was 15 mg. per cent, fasting blood sugar 85 mg. per cent, cephalin cholesterol flocculation 2+, thymol turbidity 6.0 units, total bilirubin less than 0.8 mg. per cent, total protein 4.9 gm. per cent, globulin 2.0 gm. per cent. An L.E. cell preparation was negative. Cold agglutinins were positive in a 1:20 dilution after twenty-four hours at 4°c. Lymphogranuloma venereum complement fixation test was negative. Sputum for cytology was negative on two determinations, sputum smears for acid fast bacilli were negative on four occasions. Three cultures of the sputum for acid fast bacilli and fungi revealed no growth. Two guinea pigs, inoculated with the patient's sputum, showed no evidence of tuberculosis. A culture of the bone marrow on routine, acid fast and fungus media also revealed no growth. Histoplasma skin test was negative. Purified protein derivative skin test was negative for first and intermediate strengths.

The patient was treated with 1 gm. tetracycline per day. She had a remittent fever with a temperature of about 39°c. and a relative bradycardia during the first two days of hospitalization. On the third hospital day she noticed no change in her symptoms, the fever persisted although the rales became less prominent. A repeat white blood cell count was 14,900 per cu. mm. Differential showed: eosinophils 1, bands 3, segmented neutrophils 76, lymphocytes 15, monocytes 2, atypical lymphocytes 3. On the fourth hospital day a culture of the blood revealed no growth. Roentgenograms of the chest showed no change. (Figs. 3 and 4.) The dose of tetracycline was raised to 2 gm. per day. On the following day, because of moderate dyspnea, the patient was given oxygen by mask with some relief. On the sixth hospital day her dyspnea continued with improvement on oxygen but the rales at both bases were more prominent. The febrile course continued without change. On the following day a culture of the blood revealed no growth; the sputum culture revealed moderate growth of non-hemolytic streptococci, a few alpha streptococci and a few neisseria. Sputum cultures for acid fast bacilli and a guinea pig inoculation taken on this day were subsequently negative. On the eighth hospital day a repeat roentgenogram of the chest showed progression of the bilateral generalized coalescent infiltration. The hemoglobin was 9.0 gm. per cent, white blood cell count 21,600 per cu. mm. and a culture of the sputum showed no change in flora. Treatment

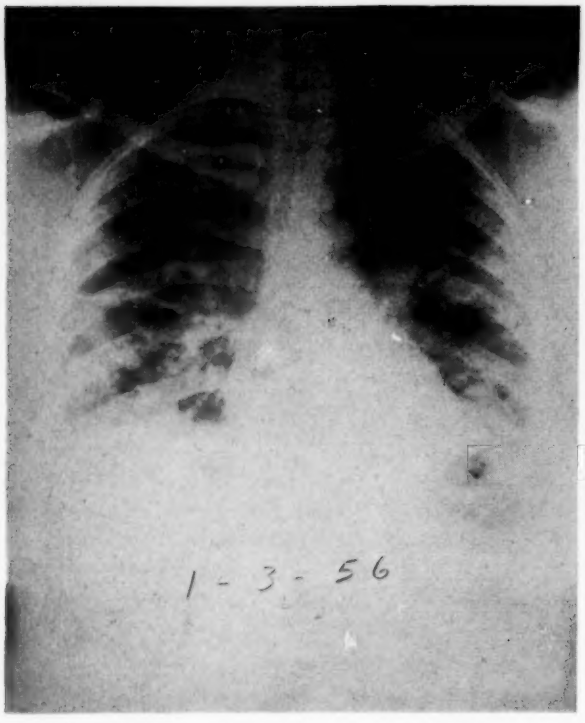

FIG. 3. Chest roentgenogram, posteroanterior view, January 3, 1956.

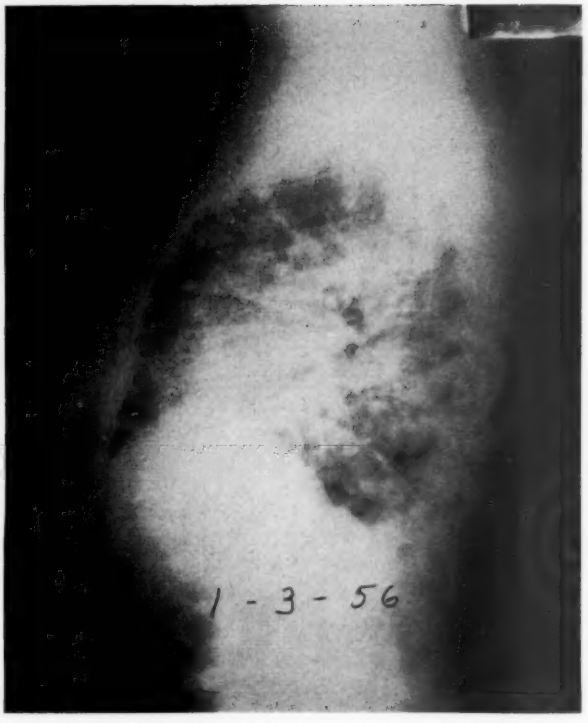

FIG. 4. Left lateral projection of chest, January 3, 1956.

was started with penicillin, streptomycin and 60 mg. prednisone per day. Tetracycline was discontinued. The temperature fell soon thereafter to approximately 36°C. where it stayed for the next twenty-four hours. On the ninth hospital day the patient coughed up a moderate amount of thick white sputum; slight cyanosis was noted even during the administration of oxygen. Moist rales were heard over the entire right chest except for the apexes, and the breath sounds were harsher at both bases. A repeat hemoglobin was 10.8 gm. per cent and the white blood cell count was 27,600 per cu. mm. Differential showed: metamyelocytes 1, bands 14, segmented neutrophils 74, lymphocytes 10, monocytes 1. She received 500 cc. of whole blood without reaction. On the tenth hospital day her temperature rose to approximately 38°C. without bradycardia. She had an episode of severe dyspnea while sitting on the commode. Physical examination was unchanged. A repeat culture of the blood again showed no growth. On the eleventh hospital day signs of consolidation appeared over both lower lung fields posteriorly; symptomatically there was no change. The white blood cell count rose to 30,500 per cu. mm.; differential showed: bands 7, segmented neutrophils 85, lymphocytes 5, monocytes 3; her hemoglobin was 12.0 gm. per cent, cold agglutinins showed no rise and a histoplasma complement fixation test was negative. Venous pressure was 142 mm. of water, circulation time was 15 seconds. Roentgenograms of the chest showed an increase in the pulmonary infiltration. (Fig. 5.) On the following day the patient appeared slightly improved; her temperature ranged between 38° and 39°C. Arterial oxygen saturation was 82 per cent without oxygen inhalation and 83.7 per cent with oxygen inhalation. A repeat white blood cell count was 32,000 per cu. mm.; differential pattern: eosinophils 5, metamyelocytes 1, bands 12, segmented neutrophils 74, lymphocytes 5, monocytes 3. Aspirated bone marrow showed no histoplasma organisms or tumor cells and was consistent with the polymorphonuclear leukocytosis. On the thirteenth hospital day a sputum examination for cytology revealed some cells suggestive but not conclusive of malignancy. Smear of the sputum stained with methylene blue and for eosinophils showed a moderate number of granulocytes and rare eosinophils. Lymphogranuloma venereum complement fixation test and a heterophil agglutination reaction were negative. A culture of the stool revealed no pathogens.

On the fourteenth hospital day the patient appeared worse, the fever persisted, but no signs of congestive failure appeared. The serum agglutinins were negative in all dilutions for typhoid O and H, paratyphoid A and B, brucella, P. tularense and proteus OX 19. Complement fixation reactions for Q fever, epidemic and murine typhus were negative. On the fifteenth hospital day the temperature rose to 39°C. and shallow respirations at a rate of 50 to 70 persisted. Signs of consolidation were noted over both upper and lower lung fields. After premedication with thorazine® and pentobarbital she was given 40 mg. of nitrogen mustard intravenously without immediate untoward effect. About eight hours later, the patient was found unresponsive with an unobtainable blood pressure, a regular apical rhythm at a rate of 100 and bubbling rales over the upper anterior lung fields that had previously been clear. She failed to respond to emergency measures and died on January 15, 1956.

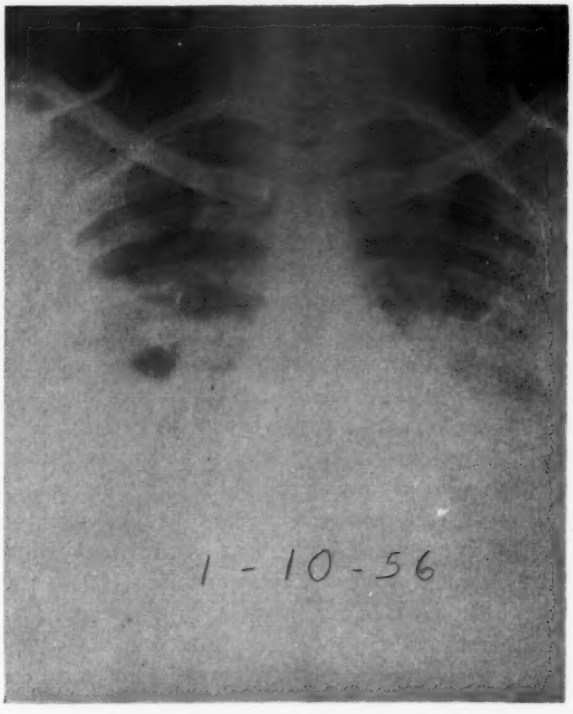

FIG. 5. Chest roentgenogram, posteroanterior view, January 10, 1956.

CLINICAL DISCUSSION

DR. SOL SHERRY: The course of this patient was fulminant and progressively downhill with death occurring on the sixteenth hospital day. The suspected diagnosis on admission was acute viral pneumonia. During hospitalization she exhibited a sustained high fever, rapidly developing signs of consolidation over both lower lung fields, increasing dyspnea, orthopnea and slight cyanosis. Cough and expectoration, although present, were never prominent. The sputum was white in color and mucoid in consistency. On one occasion the sputum was noted to contain a moderate number of granulocytes and epithelial cells. No eosinophils were seen in this material. The administration of oxygen resulted in partial relief, but did not improve the oxygen saturation of her blood. The leukocyte count rose to 32,000 per cu. mm. Tetracycline, penicillin and streptomycin therapy were ineffective. Prednisone did not alter the course of her illness. On the last day of her illness, she was given nitrogen mustard, but died eight hours later, with what appeared to be pulmonary edema. Several sputum cultures taken during the course of her illness were not helpful diagnostically. Bone marrow and repeated sputum cultures were negative, both for acid fast bacilli and fungi, as were several guinea pig inoculations. A number of blood and stool cultures were also negative. No rise in cold agglutinin titer was observed. The sputum cytology was suggestive for malignancy on one occasion and was negative on two other occasions. Skin tests for tuberculosis and histoplasmosis were negative. Complement fixation or agglutination tests were negative for lymphogranuloma, typhoid, paratyphoid, brucella, tularense, proteus OX19, Q fever and epidemic and murine typhus. The heterophil titer was negative, as was the L.E. test. Dr. Seaman, would you discuss the roentgenograms?

DR. WILLIAM B. SEAMAN: The initial examination of the chest was performed on December 31st and presented a rather diffuse interstitial infiltration, involving the lower two thirds of both lung fields. (Figs. 1 and 2.) The apical portions of both lungs seemed relatively clear. In several areas, there were ill defined radiolucencies better seen on the lateral film, suggesting multiple cavitations deep in the lung fields. No air fluid levels could be demonstrated. There were several other smaller radiolucent areas suggestive of either small cavities or emphysematous bullae. There was no definite evidence of pleural effusion on either side and the heart was within normal limits. An examination of the chest on January 3, 1956 showed very slight but definite progression in the pulmonary

infiltrations. (Figs. 3 and 4.) At that time several of the areas of infiltration, particularly in the right lower lung field, had a rounded appearance and were somewhat suggestive of a metastatic neoplasm. The diaphragms were equal in height and there was again no evidence of pleural effusion. The lateral chest film, taken on the same day, showed multiple radiolucent areas particularly in the upper lobes, but also several in the lower lobes, suggestive of cavitation, although again no air fluid levels could be demonstrated. The last examination was on January 10th and revealed an apparent increase in the consolidation of the lower half of the lung fields. (Fig. 5.) In part this was due to under exposure and in part to the high diaphragms and poor aeration. However, even accounting for this, I believe that there was some progression of the process with marked consolidation in the lower lung fields. This pattern is non-specific but it suggests more strongly an inflammatory rather than a neoplastic process. The question of lymphangitic infiltration was raised, but at no time was there any hilar adenopathy which is usually present with lymphangitic carcinoma. The presence of questionable cavitations suggested tuberculosis, but in view of the relative freedom of the upper lobes from disease, I believe this diagnosis to be unlikely. The best we can do is to interpret these films as representing a pneumonic episode with questionable cavitation probably due to a necrotizing pneumonitis.

DR. SHERRY: Would you like to suggest a specific diagnosis?

DR. SEAMAN: My guess would be a bacterial pneumonia of a necrotizing type.

DR. SHERRY: Dr. Bercu, on the basis of the data available, would you give us your interpretation of what is happening physiologically in her lungs.

DR. BERNARD BERCU: This patient had a marked decrease in pulmonary function and it is obvious that she had pulmonary insufficiency which was causing her dyspnea and cyanosis. The arterial oxygen was low and did not rise with oxygen breathing so that one could be fairly certain that she had functional A-V shunts in the lungs. In other words, she had vast areas of her lungs which were not being aerated but were being perfused by blood. This does not help too much except that it tends to eliminate consideration of the so-called alveolar capillary block syndrome since in this situation the hypoxia is usually improved with oxygen therapy. In summary, one can only confirm what Dr. Seaman has said; that she had massive infiltration in her lungs.

DR. SHERRY: On the wards, the question of an alveolar capillary block was seriously considered. It was felt that the lack of response to oxygen was in favor of an alveolar capillary block. Dr. Zimmerman, what is your opinion?

DR. HERBERT ZIMMERMAN: I would like to point out that the oxygen levels were determined while the patient was breathing oxygen through a plastic face mask. The concentration of oxygen which a patient receives through one of these masks is difficult to evaluate at the present time. Her arterial oxygen level may not have risen because an insufficient concentration of oxygen was administered. I believe we cannot completely exclude the possibility of an alveolar-capillary defect in this patient.

DR. SHERRY: The differential diagnosis to be entertained in this particular patient may be summarized as follows: (1) The patient had a pneumonia. The etiology of the pneumonia may have been bacterial, of the type that staphylococcus, streptococcus or tuberculosis might produce; or viral; or mycotic, most likely blastomycosis, coccidiodomycosis, or perhaps moniliasis. (2) Vascular disease of the lung. We may consider recurrent pulmonary emboli or periarteritis, perhaps of the Wegener granulomatosis type. (3) Malignancy. Alveolar cell carcinoma or pulmonary adenomatosis would be most likely. Also to be considered are Hodgkin's disease or a diffuse metastatic carcinoma. (4) Acute interstitial fibrosis of the Hamman-Rich type. (5) Bronchiolitis fibrosa obliterans, and (6) other granulomatous diseases of the lung. Dr. Goldman, do you believe that this list comprises a fair representation of the diagnoses to be considered?

DR. ALFRED GOLDMAN: Yes, we considered most of these diseases during her illness.

DR. SHERRY: You saw this patient in consultation. Would you continue the discussion, stressing particularly the differential diagnosis?

DR. GOLDMAN: The acute onset of chills and fever a few days before admission to the hospital with a spreading inflammatory process in the lungs, increasing leukocytosis, and failure to respond to tetracycline, penicillin, and streptomycin led us to consider, first, a bizarre type of pneumonia. Viral pneumonia was regarded as the most likely diagnosis although we are not accustomed to seeing viral pneu-

monia as a fatal disease. The pneumonia that follows influenza is usually a secondary pneumonia with a staphylococcal infection. Primary atypical pneumonia, which this patient's course could represent in so far as the progression of the pulmonary involvement is concerned, is usually associated with leukopenia, and does not have a grave prognosis. Occasionally, leukocytosis may occur, though it is not very common. The fact that the cold agglutinin titer remained at 1 to 20 and did not rise would be against a diagnosis of primary atypical pneumonia. Staphylococcal infections of the pulmonary parenchyma characteristically produce abscesses of the lungs as the disease progresses. This patient did not have the purulent sputum which would be expected in staphylococcal pneumonia. Pleural reaction is also fairly common in this variety of pneumonia. Finally, staphylococcal pneumonia usually follows another disease like influenza or occurs in a debilitated individual. Friedlander's infection of course was seriously considered. Against a diagnosis of Friedlander's pneumonia was the observation that the upper lobes were spared. Also the fact that the sputum was not purulent, did not contain blood and was not the so-called "currant jelly"-like sputum, would be somewhat against a Friedlander's pneumonia. The remainder of the clinical findings would however be consistent with this diagnosis. In both staphylococcal and Friedlander's infection the failure to respond to antibiotics is not unusual. Staphylococcus infections, notoriously, may be resistant to most of the medications that were tried. I notice in the protocol that erythromycin was not used here. Friedlander's pneumonia also has a bad prognosis in spite of anything that can be given the patient, so that both of these types of pneumonia should be seriously considered. Other types of resistant bacteria, such as the coliform and proteus groups might be considered, but are usually found in the lung after prolonged chemotherapy and antibiotics administered for other diseases. This patient was apparently healthy prior to her present acute illness.

Tuberculosis was thoroughly considered since she was a Negro woman with a febrile lung disease. The course, however, was rather acute for tuberculosis. Patients who die of tuberculosis usually have a more chronic type of clinical course unless there is a complicating hemorrhage. While leukocytosis may occur, the only times I have seen this high a count in tuberculosis have been in patients with a so-called leukemoid reaction. She had a negative tuberculin reaction which might be explained on the basis of anergy in a very seriously ill patient. On the whole, I would be against the diagnosis of tuberculosis. Mycotic infections were also considered. Most of the pulmonary mycoses are associated with chronic lesions elsewhere such as carcinoma, lymphoma or tuberculosis or in individuals who have had long term chemotherapy, antibiotics or steroid therapy. This patient did not have such a lesion, nor did she receive such therapy. Acute mycotic diseases that might have caused death in two and a half or three weeks are relatively rare. Blastomycosis may occasionally have a rapid downhill course. Dr. Seaman pointed out possible abscess cavities in the lungs which could be consistent with a fungus infection, but in general fungus infections rarely spread through the lung and cause death in two or three weeks.

Multiple pulmonary emboli can give a bizarre picture with fever, leukocytosis, dyspnea and cyanosis. Emboli are of course seen more often in the debilitated individual with cardiac disease which this patient did not have. There was no evidence that she had any peripheral phlebothromboses, although such lesions can be missed. The emboli would have had to be multiple. Furthermore there was no history of sudden chest pain with brisk pulmonary bleeding or any evidence during life of pleurisy or pleural effusion. The area of infarction would have to be large to produce the degree of fever noted in this patient.

We also considered periarteritis nodosa, but she had no eosinophilia, kidney lesion or hypertension. In Wegener's type of granulomatosis, there is usually evidence of an acute inflammatory process of the nose, with ulceration and sinusitis as well as kidney lesions. As far as malignancy is concerned, alveolar cell carcinoma of the lung or bronchiocarcinoma or pulmonary adenomatosis, which are probably all one and the same thing, do present diffuse pulmonary lesions of the nodular type as well as of the bronchopneumonic type and lobar consolidation. In this particular case the process was so acute that I believe we may rule out that type of lesion. As we well know, the lymphomas may produce lung lesions, cause fever and follow a very confusing course. We have no evidence of lymphoma since there were no nodes in the

mediastinum or adenopathy elsewhere in the body. So far as metastatic carcinomatosis is concerned, the roentgenograms do not look like the lymphangitic type. I doubt that this clinical course could be caused by carcinomatosis, but of course we do know that fever and a Hamman-Rich syndrome may occur particularly in the lymphangitic type.

The next disease under consideration was acute interstitial fibrosis of the lung. This patient had evidence of an acute interstitial disease of the lung, producing a Hamman-Rich syndrome, in view of her tachypnea, cyanosis and the character of her sputum. Acute interstitial fibrosis of the lung, which is being recorded with increasing frequency since Hamman and Rich first described it in 1935, is a disease that as a general rule is not usually associated with fever except terminally. It is a disease that is of subacute or chronic nature. I know of no cases of this disease in which death occurred so quickly except in cases where steroid therapy was given and then withdrawn. This phenomenon was reported recently in three cases and the authors attribute the deaths to the withdrawal of steroid therapy. The acute onset, short duration of the disease, fever and leucocytosis are against the diagnosis of acute interstitial fibrosis. The diagnosis of bronchiolitis fibrosa obliterans was considered. In this disease there is always some specific etiologic factor such as influenza, or the inhalation of highly irritant fumes, or a severe pulmonary infection. We do not have the evidence of such factors here and the course seems to be rather acute for that type of lesion. Other granulomatous diseases such as sarcoidosis were also considered. This lesion is a chronic one which can produce evidence of an alveolar capillary block. Sarcoidosis can be ruled out for many other reasons.

With the information that we have, particularly because of the onset and the leucocytosis, I would assume that this patient had a bacterial pneumonia of an acute interstitial type at the start. Subsequently signs of consolidation developed, indicating that the alveoli were involved with a pneumonic process or were filled with fibrin. Which of the bacterial agents could be responsible, I am unable to state. Mycotic infection would be possible although unlikely.

DR. SHERRY: How often would one have a bacterial pneumonia in which multiple sputum cultures would not reveal the responsible organism?

DR. GOLDMAN: Certainly it would be rare not to be able to pick up Friedlander's bacillus or staphylococcus which would be the two organisms that we would be mostly concerned about.

DR. SHERRY: Dr. Goldman, you did not believe that a needle biopsy of the lung or bronchoscopy was advisable in this individual. What were your reasons?

DR. GOLDMAN: The patient was quite sick. She was dyspneic and cyanotic. I thought the suggestion of a lung puncture was a good one, but I was afraid to do it because we would be apt to get a pneumothorax. A pneumothorax added to her respiratory difficulties might well cause sudden death.

DR. SHERRY: Dr. Harford, when you saw this patient, you thought that the most likely diagnosis was a primary atypical pneumonia. Do you still feel that way? Is there anything that you would like to comment upon concerning the bacteriologic or viral diagnosis in this patient.

DR. CARL HARFORD: First, I should like to comment on primary atypical pneumonia. This patient had signs of consolidation. Although it is unusual, consolidation does occur in primary atypical pneumonia. In one series of sixty-seven cases of unusual atypical pneumonia, consolidation occurred in 24 per cent.* Similarly, leukocytosis is uncommon but in the same series of sixty-seven patients, leukocyte counts of 35,000 and 39,000 per cu. mm. developed in two patients. As regards the diagnosis of a bacterial pneumonia, one would have to assume one of two things. One is that the pneumonia was so overwhelming, that even though the organism was sensitive, the pneumonia was not controlled. That seems a little unlikely to me. The other possibility would be that the patient had a bacterial pneumonia due to an organism not susceptible to the drugs used. Under these circumstances I should suspect not only that the sputum would have shown a positive culture, but that some one of the many blood cultures would have also been positive. You have already pointed out that the organisms isolated from the throat and sputum were not ones ordinarily considered pathogenic.

DR. SHERRY: Coughing and expectoration were very minimal features in this patient's course. Is that against viral pneumonia? Cer-

* Jordan, W. S., Jr., Albright, R. W., McCain, F. H. and Single, J. H. Clinical variations in primary atypical pneumonia. *Am. J. Med.*, 10: 3, 1951.

tainly 97 per cent of patients with viral pneumonia have a prominent cough.

DR. HARFORD: That is a matter of degree. Ninety-seven per cent in the series which I mentioned also had a cough. This patient had a cough, but the question is whether she had enough cough to be significant. In my experience the amount of coughing that this patient had is consistent with either bacterial or viral pneumonia. In fact, I have seen pneumococcal pneumonia with no cough at all.

DR. SHERRY: Dr. Harford, you stated that the best working diagnosis was viral pneumonia. You advised against the use of steroid therapy in this patient who was doing very poorly. You said you did not believe it would be beneficial and that it might be very harmful. Why did you believe it could be harmful?

DR. HARFORD: I believe there is evidence that the steroids can potentiate not only bacterial and fungus infections, but also viral infections.

DR. SHERRY: Dr. Flance, what are your thoughts in regards to this case?

DR. JEROME FLANCE: I would like to return to the diagnosis of interstitial fibrosis of the lung. The entire clinical course of this patient is certainly compatible with this diagnosis. We do not have a good base line on this patient so that it is difficult to determine whether the final illness represented an acute termination of a more chronic process or acute progressive lung disease. The physical signs are compatible with an interstitial lesion in that initially she did not have signs of frank consolidation but had slightly impaired percussion, normal breath sounds and a few rales. Only terminally did signs of frank consolidation develop. It is interesting to note that cells suggestive of malignancy were seen in the sputum. It is probable that in this patient changes developed in her alveolar lining cells so that these became more cuboidal which accounts for the difficulty in differentiating them from cancer cells in the sputum. The change in the alveolar lining cells is compatible with the Hamman-Rich syndrome although it is a non-specific type of reaction. The leucocytosis, fever, dyspnea and cyanosis and the remainder of the clinical course are all compatible with the diagnosis of acute interstitial fibrosis of the lung.

DR. SHERRY: Is that the diagnosis you wish to make?

DR. FLANCE: Yes.

DR. SHERRY: What is your opinion, Dr. Moore?

DR. CARL MOORE: I do not believe one needs to consider seriously Hodgkin's disease or any of the malignant lymphomas. These diseases cause pulmonary parenchymal lesions, of course, but there is nothing else to support their possible presence here. One is tempted to think that the clinician who suggested the use of nitrogen mustard may have done so with the thought of treating a possible carcinoma of the lung rather than a lymphoma.

DR. SHERRY: What about malignancy of the lung in general?

DR. MOORE: Malignancy of the lung would seem to be less likely than a viral pneumonitis or acute interstitial fibrosis, but I believe malignancy remains an outside possibility. There are two things that bother me about calling this disease an acute process: (1) the low plasma proteins, and (2) the edema which she had at the onset of her present illness. None of the diagnoses which have been suggested so far explain these two manifestations.

DR. BERCU: I would like to support what Dr. Flance said about the possibility of an acute interstitial fibrosis of the lung. The presence of cyanosis and marked hypoxia with no response to oxygen would be in keeping with this diagnosis at a stage when a great deal of lung tissue had been replaced by the vascular process. I would concur with this diagnosis.

DR. SHERRY: What do you think, Dr. Harrington?

DR. WILLIAM HARRINGTON: The finding of 5 per cent eosinophils in the blood of a patient with a white cell count of 32,000 per cu. mm. is seldom seen in patients who have an active, uncontrolled infectious disease but is in keeping with other etiologies, particularly malignant diseases. I would be surprised if this patient had any infectious process in her lungs.

DR. SHERRY: Do I understand that this means you favor a malignant disease?

DR. HARRINGTON: No, but I would favor a non-infectious process in the lungs and if you want me to be specific I would consider either alveolar cell carcinoma or the Hamman-Rich syndrome as being most compatible with her clinical and hematologic findings.

DR. SHERRY: The house staff diagnosis was viral bronchial pneumonia, suspected, acute interstitial pneumonia due to unknown organ-

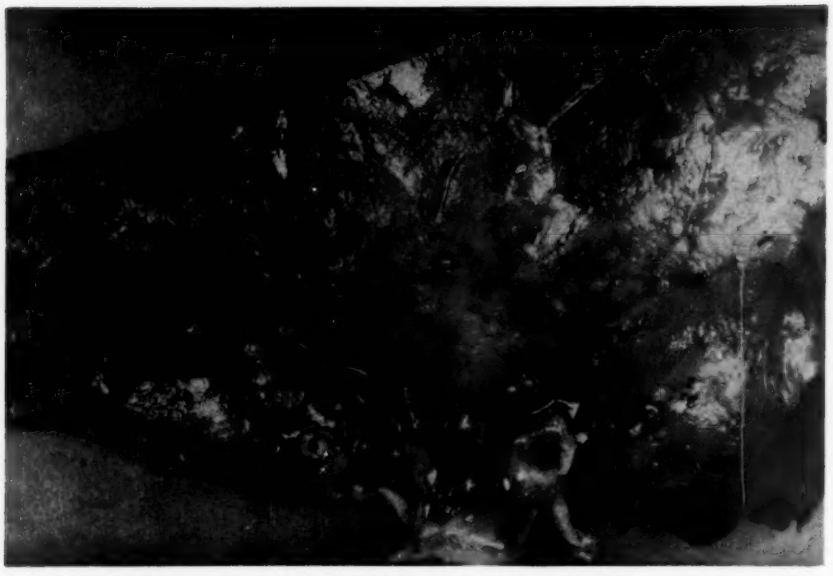

FIG. 6. Gross photograph of the cut surface of the left lung. The lower lobe, which is more extensively involved, is firm and fleshy.

isms suspected, and malignant tumor of lung, suspected. My own diagnosis is acute interstitial pneumonia of unknown etiology with secondary acute interstitial or bronchiolar fibrosis. The presence of organization would account for the lack of cough or expectoration and the progression of pulmonary insufficiency during the course of the disease. Because of the fulminating character of the disease, I would also question whether there may have been some associated pulmonary vasculitis.

PATHOLOGIC DISCUSSION

DR. WALTER C. BAUER: The external examination of this obese middle aged Negro woman was negative. On opening the chest, the lungs did not collapse, but filled the pleural cavities, and when removed from the body they maintained their shape except for the apexes of both upper lobes and the apical segments of both lower lobes, which were somewhat collapsed. The lungs together weighed 1,900 grams. In focal areas, a thin film of fibrin covered the pleural surfaces and a mottled red appearance to the underlying lung parenchyma could be seen through the pleura. Little aerated lung tissue could be palpated, except in the apical regions of both the upper and lower lobes.

The lungs cut with a consistency very much like that of liver, and maintained their shape without collapse (Fig. 6). Near the hilar regions, the lungs were homogeneous and firm and composed of a pinkish white tissue in which tiny white firm flecks could be seen. These flecks bore no constant relationship to the bronchi. In the mid-zone of the lung extending almost to the pleural surface, the surface lost the white flecks, became more fleshy in color and took on a definitely granular or porous appearance.

Minute holes were seen bordered by pink tissue. In focal areas, the consolidation extended to the pleural surface, particularly in the upper lobes, but in general, the subpleural lung tissue was boggy and somewhat crepitant.

Although the cut surface of the lungs appeared moist, only in the apical regions could thin edema fluid be expressed. Areas of hemorrhage, necrosis or cavitation were absent. Cultures of the lung for bacteria, fungi and tubercle bacilli proved to be sterile.

The tracheobronchial tree revealed only a slight amount of mucus in the major branches with minimal reddening of the respiratory mucosa. The hilar lymph nodes were only slightly enlarged, and on section, small lymphoid follicles could easily be seen.

A few recent thromboemboli were discovered in small branches of the pulmonary arteries to the lower lobes, but these were not associated with gross infarcts.

The remainder of the viscera showed very few changes. There was slight arteriolonephrosclerosis and a slightly enlarged heart weighing

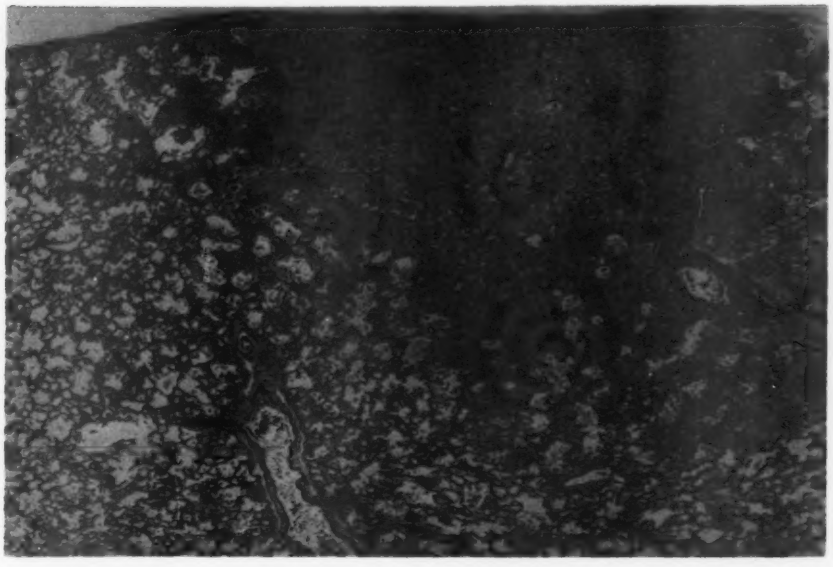

FIG. 7. Low power photomicrograph of a focal sub-pleural area of pneumonitis in the left upper lobe. The surrounding pulmonary parenchyma is normal. Hematoxylin and eosin; original magnification, × 10.

400 gm. There was an incidental leiomyoma in the stomach.

DR. ROBERT M. O'NEAL: Microscopically, only the pathologic changes in the lungs were of significance. The multiple small pulmonary thromboemboli were all recent and had apparently been present for only the last few days. There was one small microscopic recent infarct, but we do not think that the pulmonary emboli had anything to do with the majority of the pulmonary pathologic changes. Figure 7 demonstrates the patchy character of the process; it was more nearly confluent in the lower lobes. Relatively normal pulmonary parenchyma with only slight congestion surrounded an active zone of the disease process and deep within the area involved is dense fibrosis due to intra-alveolar organization of exudate into fibrous balls filling alveoli. Some of the components of this process, from the more active zone into the older, apparently quiescent, area are shown in Figures 8 through 11. In almost every diseased area, the alveolar walls were thick, but the thickening appeared to be principally the result of capillary dilatation and the increased number of open capillaries present. Figure 8 demonstrates the proteinacious, fibrinous exudate in alveoli. The alveolar lining cells were metaplastic, or had become more cuboidal, and the atypicality of their nuclei might even have been confused with the stigmata of malignancy on cytologic examination. The thickened alveolar walls contain increased numbers of cells, mostly lymphocytes, but with a few polymorphonuclear leukocytes, evidence of an interstitial component to the inflammatory process. The interstitial space in the lungs has been demonstrated by electron microscopy and probably has been expanded in this process by the presence of inflammatory cells. These alveoli were approximately half their normal size, and the alveolar ducts nearby were dilated. It seems likely that part of the apparent thickening of alveolar walls was due to a symmetric collapse of alveoli, widening the alveolar wall by a relative increase in the number of capillaries. The distended alveolar ducts associated with this process are perhaps compensatory to alveolar collapse. Figure 9 demonstrates the hyaline membrane that was present in some areas. Polymorphonuclear leukocytes were present, but these never appreciably collected in the air spaces as is so common in bacterial pneumonias. Figure 10 demonstrates the intra-alveolar exudation of fibrin that was a striking part of the pathologic picture. Figure 11 shows a less active area, in which fibrous balls, representing organized exudate, fill the air spaces. The changes are consistent with those that Hamman and Rich described in their patients as "acute diffuse interstitial fibrosis of the lungs."* However, there is no certain

* HAMMAN, L. and RICH, A. R. Acute diffuse interstitial fibrosis of the lungs. *Bull. Johns Hopkins Hosp.*, 74: 177, 1944.

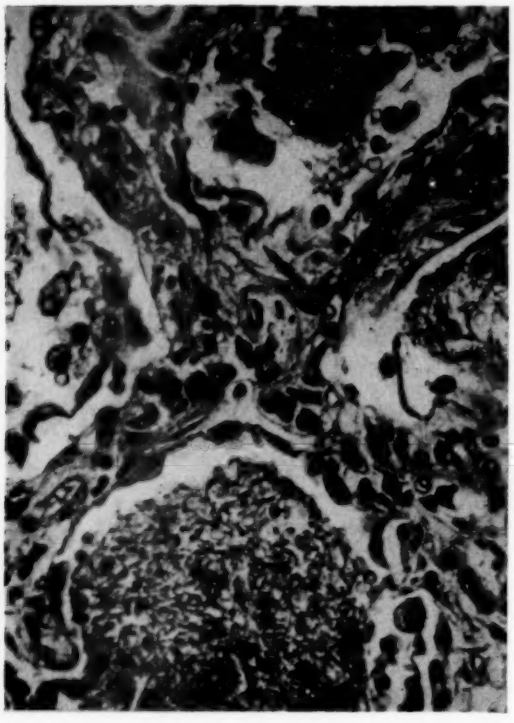

FIG. 8. Thickened alveolar walls containing inflammatory cells. The prominent, metaplastic alveolar epithelium is artefactually elevated. Heidenhein's stain, × 300.

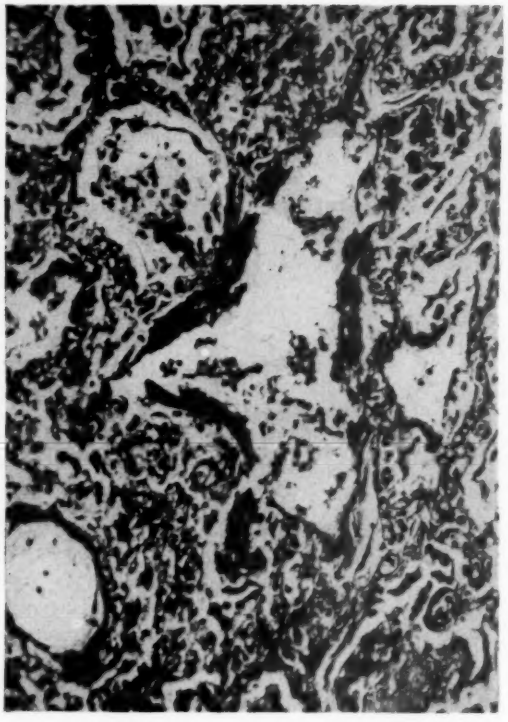

FIG. 9. A discontinuous hyaline membrane is adherent to the wall of an alveolar duct. Periodic acid-Schiff, × 125.

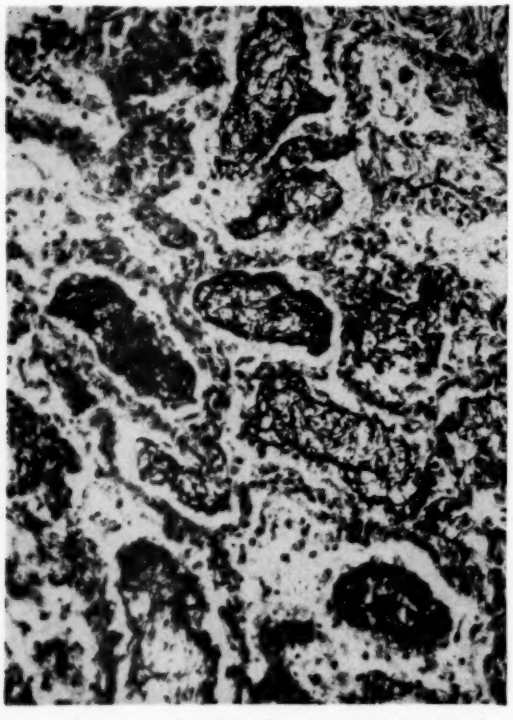

FIG. 10. Intra-alveolar clumps of fibrin fill the air spaces. Phosphotungstic acid-hematoxylin and eosin, × 140.

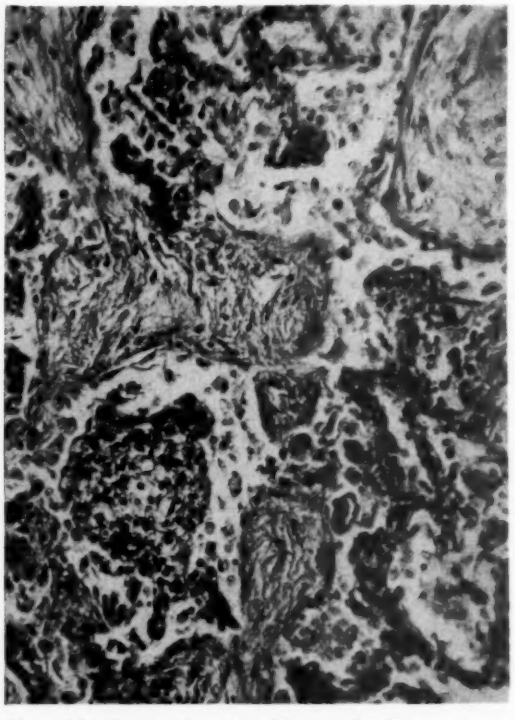

FIG. 11. Intra-alveolar fibrous bodies occupy peripheral air spaces. van Gieson stain, × 130.

evidence that this is a single specific disease. The pathologic picture is non-specific.

Final anatomic diagnoses: Primary: "interstitial pneumonitis" of unknown etiology with organization of intra-alveolar exudate, most widespread in the lower lobes; fibrinous pleuritis, bilateral, slight; multiple recent thrombi in small pulmonary arteries; recent small pulmonary infarct. Accessory: generalized slight arteriosclerosis; hypertrophy of the heart, 400 gm.; leiomyoma of the stomach; cholesterolosis of the gall bladder; cholelithiasis, solitary cholesterol stone.

Acknowledgment: Gross and microscopic photographs were supplied by the Department of Pathology, Washington University School of Medicine.

Case Reports

Bronchopneumonia, Empyema, Pneumothorax and Bacteremia Due to Salmonella Choleraesuis (Var. Kunzendorf) Treated with Chloramphenicol*

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ALTHOUGH the incidence of bronchopneumonia in the course of Salmonella choleraesuis infection has been well documented, the occurrence of empyema is extremely rare and hydropneumothorax apparently has not been recorded as a complication. There is no record in the literature of successful treatment of such rare complications. These circumstances would seem to warrant the present report.

Localization of S. choleraesuis infection in the pulmonary system in the course of generalized sepsis with this organism has been described by several writers. In 1928 Bullowa[1] reported a case of lobar pneumonia due to Salmonella suipestifer, verified by blood and sputum cultures, and by postmortem examination. There was no pleural effusion.

In 1936 Cohen, Fink and Gray[2] reported the occurrence of pleural effusion with pneumonitis and pericarditis in the course of S. choleraesuis infection. The pleural effusion, however, was sterile and apparently was a transudate. Harvey[3] in 1937 analyzed fifty cases of proved S. suipestifer infections from the literature, to which he added twenty-one cases seen at the Johns Hopkins Hospital. Of the seventy-one patients, twenty-four had evidence of pulmonary or pleural involvement, the great majority having bronchopneumonia. One of the patients in his own series had pleural effusion. This patient, however, had myocardial insufficiency and the pleural fluid was sterile. Harvey mentioned Boller (1930) and Van der Hoeden and Hulst (1933) as recovering the organism from the pleural effusion of their respective single patients. Goulder, Kingsland and Janeway[4] in 1942 stated that bronchopneumonia develops in 30 per cent of patients with S. choleraesuis infection. They also pointed out that pleural effusion is a rare finding. In 1943 Seligman, Saphra and Wasserman[5] analyzed 1,000 patients with salmonella infections. They found that ninety cases were due to S. choleraesuis, bronchopneumonia developed in eight patients and pleurisy in four. No mention is made of empyema. In 1946 the same authors[6] published an analysis of 2,000 salmonella infections of which 144 were due to S. choleraesuis. In five of the latter pulmonary involvement was present, mostly in the form of bronchopneumonia. Again, empyema is not mentioned.

The treatment of S. choleraesuis infections prior to the advent of the sulfonamide drugs had been purely symptomatic, some patients recovering spontaneously without any specific treatment.[3,4,7,8,9] The use of sulfonamides, however, proved to be of questionable value and none of the recoveries could be definitely attributed to these drugs.

In 1950 Yu and Fournier[10] reported a case of a twelve month old child with S. choleraesuis bacteremia without localization. Six gm. of chloromycetin® were administered to the child over a period of nine days with complete recovery. In 1953, Weller[11] described a case of S. choleraesuis bacteremia in a fifty year old man who was treated with chloromycetin. He received 113 gm. over a period of thirty-five days and became asymptomatic. Two weeks following discharge from the hospital the bacteremia

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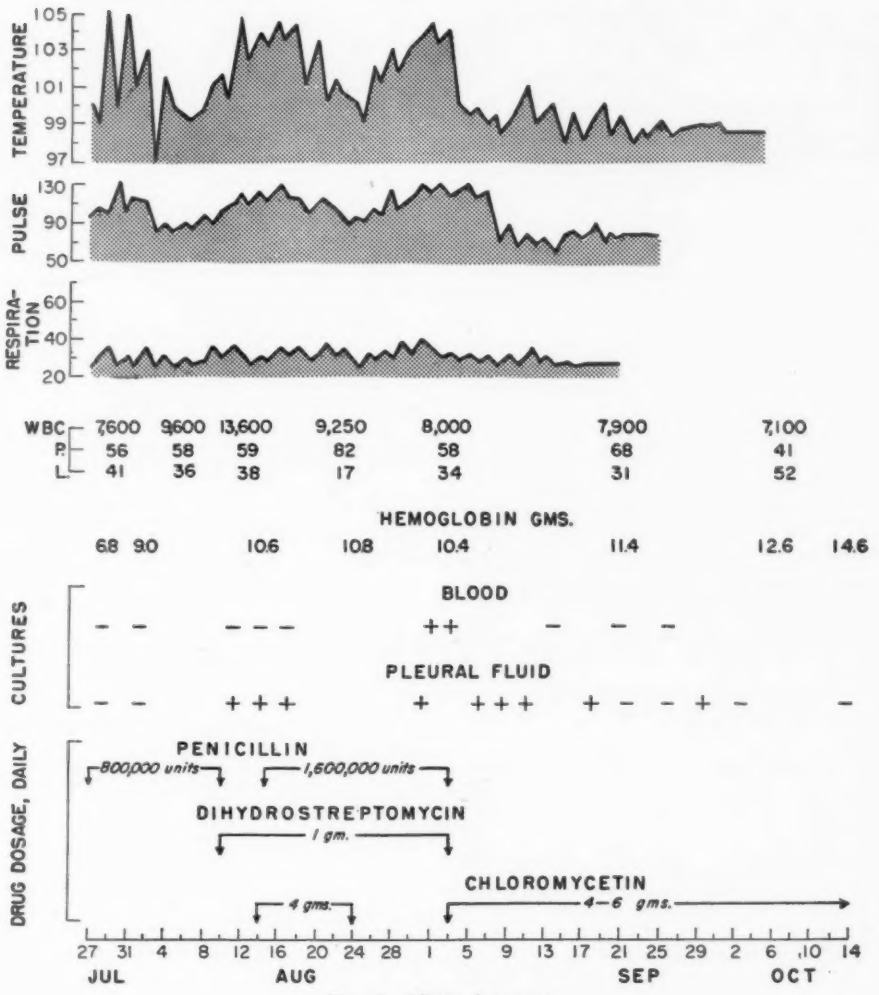

FIG. 1. Clinical course.

recurred. The patient succumbed to a ruptured abdominal aortic aneurysm. S. choleraesuis was grown from the bile in the gall bladder. In 1953 Loe Ping Kian et al.[12] reported the result of treatment of 100 cases of salmonella infections with chloromycetin. However, none of these included S. choleraesuis.

CASE REPORT

H. E., a twenty year old white man, was admitted to this hospital on July 27, 1949, with the main complaint of pain in the right chest of four days duration. On July 23, while walking, the patient suddenly experienced a sharp pain and a sense of oppression in the right chest. Deep inspiration or movement of the body aggravated the chest pain. The next day the chest pain subsided somewhat but he began to experience shortness of breath on mild exertion. During the next two days the patient remained in bed and upon turning from side to side felt as though "there was water in the right chest." He had a slight, dry cough; there was no hemoptysis. He felt feverish but had no chills. There was no sore throat, no abdominal pain, no vomiting or diarrhea and no complaints referable to the genitourinary system.

The patient had been in good health prior to the present illness. He had been a city dweller all his life and had not been outside New York City. Approximately seven to ten days prior to the onset of his illness he ate a cheese sandwich in a restaurant but to his recollection had no pork or any other meat products. There were no household pets, nor was there any contact with farm animals.

Past history revealed a diagnosis of bronchitis at the age of nine months. In 1945, while in the Navy, the patient had several episodes of acute bronchitis. Since 1947 he had repeated episodes of cough without expectoration or hemoptysis.

The patient's paternal parents and a cousin had pulmonary tuberculosis. However, there was no contact.

Physical examination on admission revealed a thin, frail boy who appeared chronically ill, dyspneic and orthopneic. There was no cyanosis. The pharynx and tonsils were not inflamed. No lymph nodes were

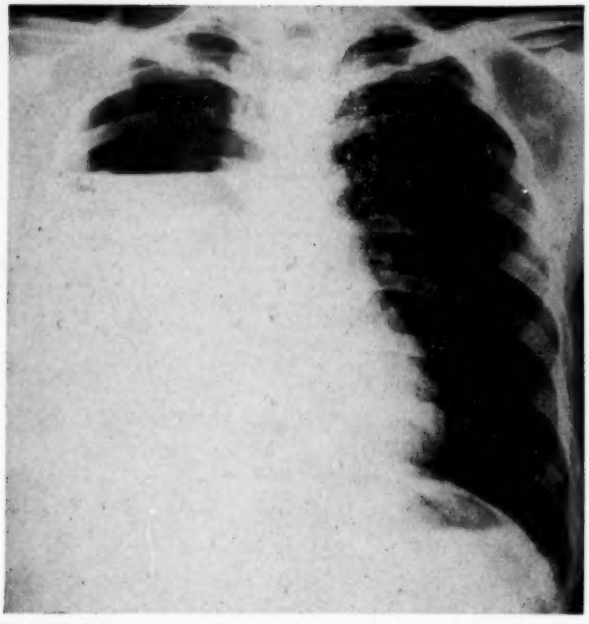

FIG. 2. Film on admission showing hydropneumothorax on the right with a fluid level at the fifth rib posteriorly.

palpable. The trachea was deviated to the left. The right chest appeared more voluminous than the left and lagged with respirations. On percussion there was flatness from the right base to the third intercostal space anteriorly and to the fifth thoracic space posteriorly. Breath sounds, vocal resonance and tactile fremitus were markedly diminished over the entire right lung field except at the apex where the breath sounds were bronchovesicular, and vocal fremitus was increased. The left lung was clear. The apex of the heart was palpable in the fifth intercostal space within the left midclavicular line. Heart sounds were of good quality; there were no murmurs or arrythmias. (Fig. 1.)

The temperature on admission was 100.8°F., pulse 84, respirations 24. The hemoglobin was 6.8 gm./100 cc., red blood cell count 2,800,000/cu. mm., the red cells showing anisocytosis and polychromasia. White blood cell count was 7,600/cu. mm., neutrophils 56 per cent, including 4 per cent band forms; lymphocytes 41 per cent; monocytes 1 per cent; eosinophils 2 per cent. Erythrocyte sedimentation rate was 26 mm. in one hour, corrected (Westergren method). The hematocrit was 32 per cent. Urinalysis showed no sugar or albumin; the sediment contained 3 to 5 white blood cells per high power field. The electrocardiogram showed a normal tracing. Throat culture showed normal flora. Culture of sputum and gastric lavage were negative for tubercle bacilli. Chest x-rays taken on admission (Fig. 2) showed a hydropneumothorax on the right with a fluid level at the fifth rib posteriorly; the left lung appeared clear; the heart was not displaced. Thoracentesis was performed and 1,600 cc. of thick, dark brown fluid was obtained which clotted on standing. A follow-up x-ray (Fig. 3) was interpreted by the roentgenologist as showing "a small amount of fluid in the lower third of the right chest; the right lung is 80 per cent re-expanded; bronchopneumonic and atelectatic zones are noted in the lower half of the right lung."

Thoracenteses were repeated whenever physical findings showed a reaccumulation of fluid. During the first two weeks two specimens of the fluid from the right chest grew out Alcaligenes fecalis on culture. This was considered to be a contaminant. Repeated smears, cultures and guinea pig innoculation did not reveal tubercle bacilli. Blood cultures during the same period were sterile. Sputum and throat cultures revealed no unusual organisms.

On July 29, the temperature rose to 105°F. and was accompanied by a chill. At this time the white blood cell count was 9,600/cu. mm.; neutrophils 58 per cent; lymphocytes 30 per cent; monocytes 10 per cent eosinophils 2 per cent. Blood culture and the pleural fluid were sterile. The patient was given penicillin intramuscularly, 400,000 units twice daily, together with supportive treatment which consisted of blood transfusion, intravenous infusions of glucose and saline solution, and parenteral vitamins. The fever gradually subsided and remained low grade until August 11 when there was another rise to 105°F. with accompanying chills. The patient appeared acutely ill and complained of pains in the right chest, shortness of breath, painful joints and a sore throat. The pharynx was markedly inflamed. Examination of the chest showed reaccumulation of fluid in the right thorax. The joints were not swollen. At this time the white blood cell count was 13,600/cu. mm., neutrophils 59 per cent; lymphocytes 34 per cent; monocytes 4 per cent; eosinophils 3 per cent. Hemoglobin was 10.6 gm./100 cc.; red blood cell count 3,800,000/cu. mm. The urine was negative for albumin and sugar; the sediment was not remarkable. Culture of urine and stool were negative for intestinal pathogens. Spinal fluid was clear and under normal pressure. Microscopic examination showed no cells. Chemical analysis was normal. Throat culture revealed Micrococcus catarrhalis. An x-ray of the chest (Fig. 4) showed reaccumulation of fluid in the right thorax; there was no evidence of pneumothorax. Penicillin was administered, 3 gm. daily for the first three days, followed by 1 gm. daily. However, the fever remained septic, reaching a peak of 105°F. daily.

On August 15 the laboratory reported that the chest fluid cultures taken three and four days previously were positive for a salmonella organism which agglutinated with salmonella group C (VI, VII) serum. This organism was identified as Salmonella choleraesuis, var. kunzendorf. The patient was then given chloromycetin in the dosage of 1 gm. every six hours, after a priming dose of 3 gm.; dihydrostreptomycin was continued. On the fourth day of this therapy the fever began to subside and the patient appeared to be more comfortable. Since the temperature had not

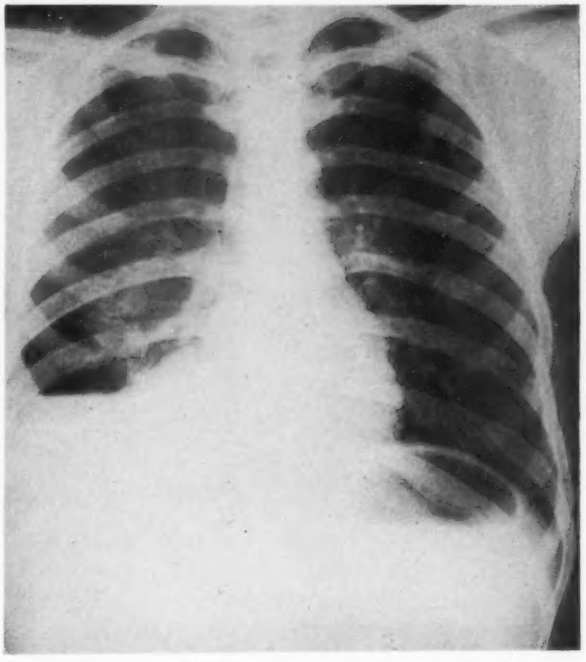

FIG. 3. Following initial thoracentesis. The right lung is 80 per cent re-expanded. There is a small amount of fluid at the right base. Bronchopneumonic and atelectatic zones are noted in the lower half of the right lung.

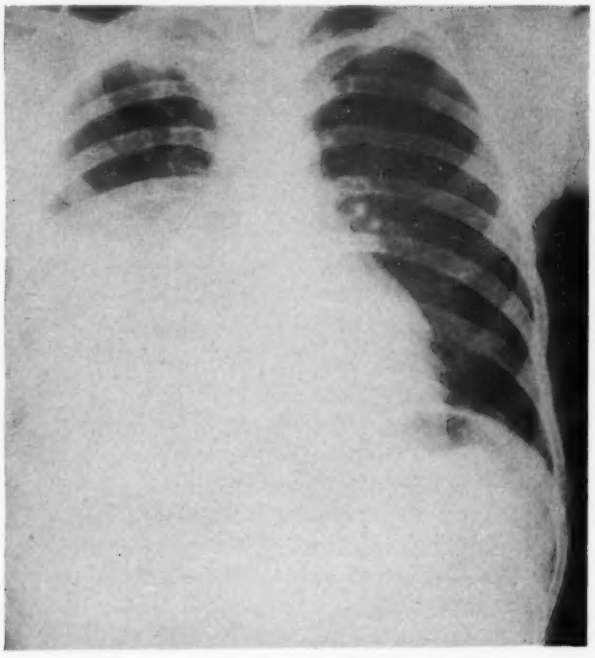

FIG. 4. Showing reaccumulation of fluid on August 11, coincident with a rise of temperature to 105°F.

TABLE 1
SENSITIVITY OF S. CHOLERAESUIS TO VARIOUS ANTIBIOTICS*

	Strepto-mycin	Chloro-mycetin	Aureo-mycin	Sulfa-diazine
Resistant to....	4	2	5	50
Sensitive to....	5	3	10	..

* Concentration expressed in gamma per cc. except sulfadiazine which is expressed in mg. per cc.

dropped appreciably, penicillin therapy was resumed, the dosage being 400,000 units every six hours. Three days later the chest and joint pains disappeared and the patient became less dyspneic. Cultures of the pleural fluid were still positive for S. choleraesuis. On August 24, after an intake of 29 gm. of chloromycetin, the temperature became normal.

At this time stomatitis, rectal papillitis and pruritus in the anal-genital region occurred. Since it was believed that these developments were due to chloromycetin, this medication was discontinued. The following day the temperature began to rise gradually and on September 1 reached 104.2°F. The patient again complained of pain in the joints, shortness of breath and chilly sensation. The chest x-ray (Fig. 5) showed reaccumulation of fluid. Blood cultures taken September 1 and September 2 were positive for S. choleraesuis. Repeated stool and urine cultures revealed no pathogens. The white blood cell count was 8,000/cu. mm.; neutrophils 62 per cent; lymphocytes 34 per cent; monocytes 3 per cent; basophils 1 per cent. Sputum culture was sterile. Agglutination tests using the patient's serum and the organism cultured from the chest fluid showed an increase in titre from 1:20 on August 15 to 1:320 on September 20. Sensitivity tests (Table 1) to the available antibiotics revealed that the organism was most sensitive to chloromycetin in concentration of 3 gamma per cc. Penicillin and dihydrostreptomycin were then discontinued and therapy with chloromycetin was resumed in the dosage of 4 to 6 gm. daily. The total dose of dihydrostreptomycin was 25 gm., that of penicillin, 19,000,000 units. Concurrently, large doses of parenteral vitamins were administered. Within five days of initiation of this therapy the temperature descended by lysis and, with the exception of an occasional rise to 101°F., remained normal during the remainder of hospitalization. Several blood cultures following resumption of chloromycetin showed no growth. However, repeated aspirations of the right chest revealed the same thick, brown fluid which did not become sterile until after September 29. A series of chest x-rays continued to show bronchopneumonic patches and reaccumulation of fluid in the right chest, thus necessitating frequent aspirations. In an effort to prevent this reaccumulation 100 mg. of aureomycin were instilled into the right pleural cavity. This procedure was discontinued when it became evident that the fluid reaccumulated more rapidly. With each subsequent thoracentesis the amount of fluid gradually diminished and after October 20 no further evidence

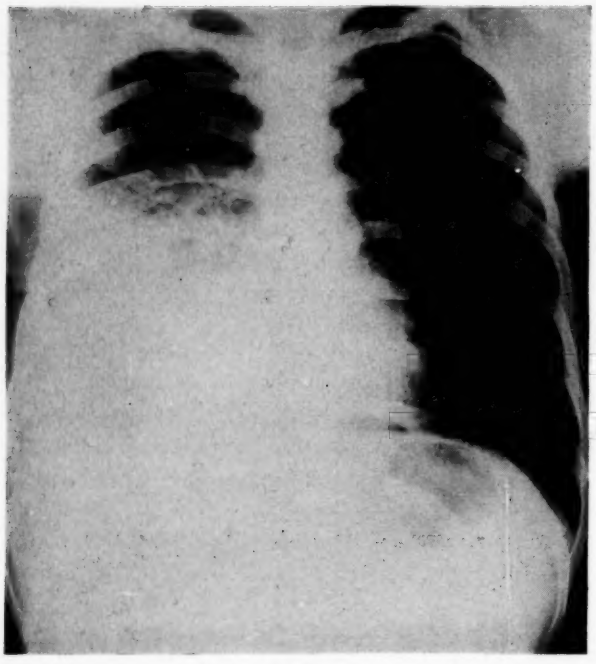

FIG. 5. Reaccumulation of fluid in the right chest on September 1. This was accompanied by blood cultures positive for S. choleraesuis.

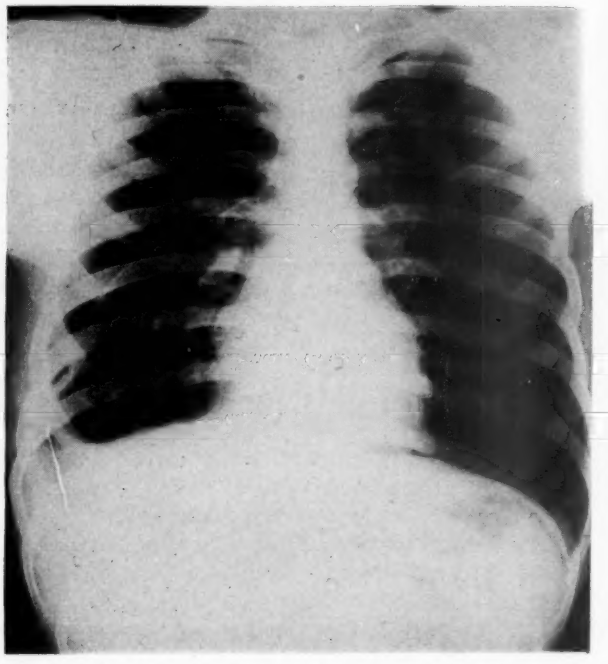

FIG. 6. October 21; showing thickened interlobar and diaphragmatic pleura in the right chest with areas of atelectasis in the right lower lobe and obliteration of the right costophrenic sinus. A similar film was obtained two years later during a follow-up examination.

of empyema was found. A chest roentgenogram on October 21 (Fig. 6) revealed thickened interlobar and diaphragmatic pleura in the right chest with obliteration of the right costophrenic sinus; there were some areas of atelectasis in the right lower lobe; there was no evidence of an exudate. The patient began to show subjective and objective improvement. Chloromycetin therapy was continued until November 2, a total of 292 gm. having been administered.

On November 16 the patient began to complain of a burning sensation in the plantar aspects of both feet. A neurologic consultant was of the opinion that the patient had a toxic neuritis, secondary to chloromycetin. The patient received large doses of vitamin B complex together with thiamin chloride. He made a gradual recovery and was discharged on December 27.

The patient was seen two years later in the follow-up clinic and found to be in excellent health. The chest x-ray at this time showed no appreciable change from Figure 6.

COMMENTS

Because of its high degree of invasiveness and pathogenicity, S. choleraesuis may produce a protean clinical picture. With the exception of Salmonella typhimurium, S. choleraesuis is the most invasive member of the entire salmonella group. The two organisms are similar in that they both have a great predilection for invasion of the blood stream but differ in that the latter is infrequently isolated from the stool. The association of septicemia with gastrointestinal symptoms is most unusual in cases of S. choleraesuis infection.[3,4,13] This organism also produces more extra-intestinal localizations than any other salmonella type.[14] Seligman, Saphra and Wasserman[5] report that of seventy-eight cases of S. choleraesuis infections, septicemia was present in thirty-eight and in twenty-four specific localization was observed. Felsenfeld,[15] Saphra[16] and Saphra and Wasserman[13] emphasize that septicemia occurs most frequently in patients in whom the clinical course is characterized by septic or typhoidal fever without any evidence of localization. Thus in analyzing 329 cases of S. choleraesuis infection, Saphra and Wasserman[13] found positive blood cultures without localization in 163 (49.5 per cent). Edwards et al.[14] isolated S. choleraesuis in 45.6 per cent of all blood cultures positive for salmonella organisms. Harvey[3] in 1937 reported positive blood cultures in 74 per cent of seventy-one cases with sporadic S. choleraesuis infection. Certain localizations, particularly bacterial endocarditis, are notoriously accompanied by septicemia.[7,8,17,18,19] Less frequently, diarrhea with fever and septic abortion (parametritis) may be associated with septicemia.[13]

The clinical course may be characterized by a septic or typhoidal fever without evidence of localization, or by extraintestinal localizations such as bronchopneumonia,[3,4,7,8] osteomyelitis,[13] osteoarthritis,[3,13] psoas abscess,[13] otitis media,[13] purulent meningitis, cystitis or pyelitis.[3,13] The infection may be primary or may occur as a complication of another totally unrelated illness such as diabetes mellitus, streptococcic throat infection, lymphatic leukemia, scarlet fever,[9] meningococcal meningitis,[20] tuberculosis, urinary tract infection, following surgery[3] or in the course of a debilitated state following fracture immobilization.[16] That S. choleraesuis was the responsible agent in these localizations was established by obtaining a culture of the organism from the pus, exudate, urine, spinal fluid or synovial fluid. However, the contention of most authors that bronchopneumonia may be due to S. choleraesuis has been based mainly upon the roentgenographic evidence of bronchopneumonia in the course of S. choleraesuis infection. Jager and Lamb[8] were the first to question the validity of this assumption. Among the six cases of S. choleraesuis infection reported by these authors roentgenographic evidence of bronchopneumonia was present in three. In two of these only pneumococci were cultured from the sputum, and in the third case pneumococci were isolated from the postmortem examination of the involved lung. They also mention Kuttner and Zepp[21] who reported a case of a two year old child with S. suipestifer bacteremia and bronchopneumonia. Pathologic examination of the involved lung revealed only pneumococci and B. influenzae. Examination of sputa of cases published in other reviews of S. choleraesuis infection[3,4,7,8] failed to reveal the organism in many showing clinical evidence of bronchopneumonia. However, definite proof that S. choleraesuis was the causative agent of the pneumonic process was obtained by Bullowa[1] and Harvey.[3] The latter found bacteriologic evidence of S. choleraesuis as the cause of bronchopneumonia in only six of a total of twenty-four cases of bronchopneumonia. More recently, Saphra and Wasserman[13] described thirty-two cases, in which the clinical features of pneumonia were observed, occurring in 329 infections with S. choleraesuis. In some of these cases positive sputum or blood cultures were noted and in some empyema or pleurisy was present. The authors, however, do not break down their cases to show in how many the pneumonic process was proved to be due to S. choleraesuis. These observations suggest that pulmonary involvement occurring during this infection may be due to secondary invaders rather than to S. choleraesuis.

The bronchopneumonia in the case cited in this report would have been of doubtful etiology in view of the failure to grow S. choleraesuis from the sputum. However, in the presence of empyema and pneumothorax prior to thoracentesis it is reasonable to postulate that the pathologic process began at the periphery of the right lung as a bronchopneumonic patch due to S. choleraesuis with subsequent rupture into the pleural cavity. Contrary to the usual course of events, invasion of the blood stream occurred following the onset of bronchopneumonia and empyema. This is evidenced by the fact that the positive blood cultures were obtained two weeks after two specimens of the empyema fluid were found to contain S. choleraesuis organism. It is also significant that prior to admission to the hospital the patient did not have a septic type of fever.

The source of infection in this case is not known. This is not surprising, since all the cases reported in several series of S. choleraesuis infections since 1937 have been sporadic, in which the source of infection could not be ascertained.[3,4,7,8] The epidemiology of S. choleraesuis infection still remains a mystery. The bacterium is found most frequently in carnivora, particularly the hog. Edwards et al.[14] found that 906 of 1,121 cultures of S. choleraesuis were from swine, the majority of cultures being isolated from the internal organs of animals affected with cholera. However, most of the sporadic cases have occurred among persons living in large cities who had no contact with hogs. Many had not eaten raw or insufficiently cooked pork. The possibility of household pets being responsible for some infections has been considered. That human carriers are not implicated is attested by the fact that healthy carriers are extremely rare. Saphra and Wasserman[13] reported positive stool cultures in three healthy individuals, while Bruner[14] reported one such case. Our patient had not been exposed to any known source of infection.

The high degree of invasiveness of S. choleraesuis is matched by its high mortality rate. The incidence of death as reported by several authors[3,5,6,14,16] ranges from 20 per cent to 41.3 per cent. This compares to a mortality rate of

5 per cent due to other salmonella organisms.[6] The incidence of fatalities is higher in patients under one year of age and in those over fifty years.

S. choleraesuis infection may occur at any age but its incidence is low under one year of age, higher between the ages of one to five years, and particularly high between fifty and sixty years. It is in the latter age group that S. choleraesuis infection frequently complicates a primary disease.

Although no definite conclusion as to the most advisable method of therapy can be drawn from one case, certain facts should be considered. The organism was found to be more sensitive to chloromycetin, in vitro, than to any other antibiotic. (Table 1.) Clinically, the temperature dropped to normal upon administration of chloromycetin, to be followed by a rise in temperature upon withdrawal of the drug, and again by a drop with its resumption. It is also of interest that the blood became sterile almost immediately after initiation of therapy. The empyema fluid, on the other hand, remained infected until September 29 despite the fact that by that time a total of 145 gm. of chloromycetin had been administered. This experience, as well as that of Weller,[11] who found S. choleraesuis organisms in the bile of the gall bladder after administration of 113 gm. of chloromycetin, leads one to the conclusion that this organism may remain resistant to a susceptible drug for a long period. One must not be misled by a normal temperature and an asymptomatic clinical course. This clinical observation corroborates Pike and MacKenzie[22] who have shown, experimentally, that certain salmonella strains may survive in organs for relatively long periods.

SUMMARY

A case of infection due to S. choleraesuis var. Kunzendorf with certain unusual clinical features, is reported.

The evolution of the clinical picture in this case differs from that hitherto described in such infections, in that the pathologic process began as a bronchopneumonia due to S. choleraesuis, with subsequent empyema, pneumothorax and septicemia. Review of the literature failed to reveal another case with such a clinical sequence.

The patient recovered following administration of 292 gm. of chloromycetin. It is pointed out that specific therapy must be administered over a long period, since this organism may remain dormant despite apparent clinical improvement.

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Tietze's Syndrome*

A Review of the Literature

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IN 1921 Tietze[1] published a report of four unusual case histories and summarized the clinical picture as follows: "We are dealing with the appearance of painful swellings in the region of the upper costal cartilages . . . that have developed in a matter of several weeks to months and have shown variations and remissions in their course." These swellings appeared neither inflammatory, granulomatous nor neoplastic in nature. Tietze referred to the lesions as a dystrophy of the costal cartilage. They have since been called "Tietze's disease," "Tietze's syndrome" and "costal chondritis." In 1952 fourteen cases apparently identical with this condition were reported by Chantraine,[2] who suggested the term "chondropathia tuberosa." Düben[3] believed that this designation was superfluous. Since the etiologic and pathologic identities of this condition remain obscure, I prefer the term Tietze's syndrome to Tietze's disease or costal chondritis.

As more than 155 cases have been reported since Tietze's article it seems that a critical review of the world literature is in order. This will be preceded by the description of a typical case which I have observed:

CASE REPORT

A sixteen year old girl was first seen in the Out-Patient Department of the Hospital of the University of Pennsylvania on January 12, 1953. She complained of "gas pains" and a "lump on the chest." She had been well until December 24, 1952, on which date she began to have sharp, intermittent pains over the upper sternum, radiating occasionally toward the right shoulder. Pain was exacerbated by coughing and deep inspiration. On self-examination the patient had found a tender swelling, the size of an egg yolk, on her chest. The swelling had not been noticed previously and there was no history of trauma to the area. Tenderness to pressure had disappeared a few days after onset and had never recurred.

Systemic review was non-contributory. There were no symptoms pertaining to the respiratory tract. Past medical history and family history were essentially negative.

Physical examination revealed a well developed, apparently well nourished sixteen year old Negro girl. She was apathetic and showed moderate retardation of thought. Temperature, pulse and respirations were normal. The blood pressure was 120/75. There was a soft, blowing pulmonic systolic murmur. Examination of other systems was entirely normal except for the following: Over the right second costal cartilage was a hard, fixed, non-tender, fusiform swelling which measured approximately 3 by 5 cm. There was no local heat or erythema. The overlying skin was freely movable and was neither ulcerated nor edematous. (Fig. 1A and B.)

The patient was examined on several occasions during the next nine months. Spontaneous pains had gradually subsided by June 1, 1953. The swelling remained essentially unchanged. No treatment was given.

Roentgenograms of the chest, including tomograms, were negative. All costal cartilages were normally radiolucent and showed no calcification. An electrocardiogram was within normal limits. In June, 1953, the hemoglobin was 12.0 gm. per 100 ml., the leukocyte count was 5,200 and a peripheral blood smear was normal. Erythrocyte sedimentation rate and repeated urinalyses were normal. Serum Kolmer and Kline tests were non-reactive. Intradermal tests with old tuberculin were negative in the 0.01 mg. and 0.1 mg. strengths.

On October 15th a biopsy of the right second costal cartilage was performed. At operation there was no evidence of inflammation or adhesions in the tissues overlying the cartilage. A moderate degree of anterior angulation of the cartilage was noted, especially at the costochondral junction. The perichondrium was smooth and not grossly thickened; the cartilage appeared normal upon section. A small wedge of

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FIG. 1A. Ventral view of the patient in July, 1953.

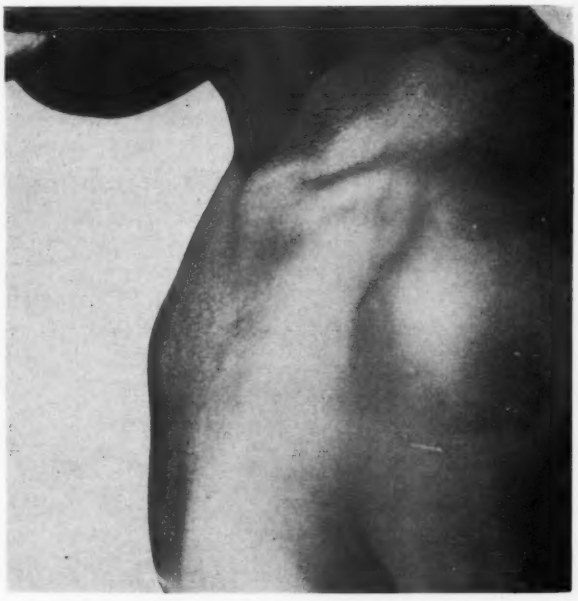

FIG. 1B. Lateral view of the patient in July, 1953.

tissue was excised, including cartilage, costochondral junction and rib. Microscopically the specimen appeared to be normal cartilage, showing no evidence of tumor or inflammation. The fragment of bone included in the specimen showed "numerous cement lines, suggesting waves of bone formation."

The operative wound healed normally. The swelling increased slightly in size during the first few months following biopsy. The patient was last examined in May, 1954, at which time the swelling was slightly more prominent than it had been when the patient was photographed. There has been no recurrence of pain since the biopsy was performed.

REVIEW OF THE LITERATURE

A search of the world literature through May, 1954, has disclosed 159 cases which are compatible with the clinical picture of Tietze's syndrome. Since some of these case reports are substantiated by only scant clinical data, this series might well include cases of undiagnosed neoplasms, infections, etc., of the costal cartilage. The diagnosis of Tietze's syndrome, I believe, can be proved only by biopsy. Therefore all biopsied cases have been studied in more detail. In Table I the roentgenologic and pathologic findings of all biopsied cases have been copied or paraphrased essentially *in toto* from the original articles or translations thereof.

Incidence. The fact that only 159 cases were found in the literature over a twenty-three-year period would indicate that Tietze's syndrome is a rare entity. Of these cases only seven have appeared in North American journals.[4,5] Since many physicians may not be aware of this syndrome, many cases probably are either unrecognized or unreported; therefore the rarity of the syndrome may be more apparent than real.

For example, Geddes[6] could report on twenty-two patients during a three-year period and mentions eight others whose records were lost. An orthopedic surgeon has, within the past four years, seen at least three cases of unexplained, painful swelling of the costal cartilage among student nurses at a Philadelphia hospital of modest size.[7] The literature contains occasional reports of undiagnosed costal cartilage swellings, some of which may have been Tietze's syndrome.[8–12]

Etiology. Predisposing factors: The patient's age was given in eighty-three cases. The mean age is 32.3 years; the median, twenty-eight years. The range is from eleven to seventy-nine years. Thirty patients were in the third decade of life. (Fig. 2.) Of the biopsied cases, the age was stated in fourteen. The mean age is 28.8 years, with a range from eleven to fifty-seven years. The sex of the patient was stated in 119 cases; there were fifty-nine males and sixty females. As to race, the syndrome has been seen largely in Western European and in Japanese subjects; the patient reported on herein is an American Negro. Tietze's syndrome appears to have no occupational preference. Patients with many diverse occupations have been seen.[6,13–15]

Although nutritional deficiencies were implicated etiologically in earlier reports,[1,16,17] the data now available do not support this viewpoint. A statement as to clinical nutritional status was found in sixty cases. Forty-eight of these patients were said to have a normal nutritional status; twelve were mal- or undernourished. Among six biopsied cases in which data were given, five patients were considered adequately nourished. Evans and Eames[18] believed that the syndrome was a local manifestation of vitamin B and C deficiencies. Guarner[19] suggested the possibility of deficiency in certain amino acids.

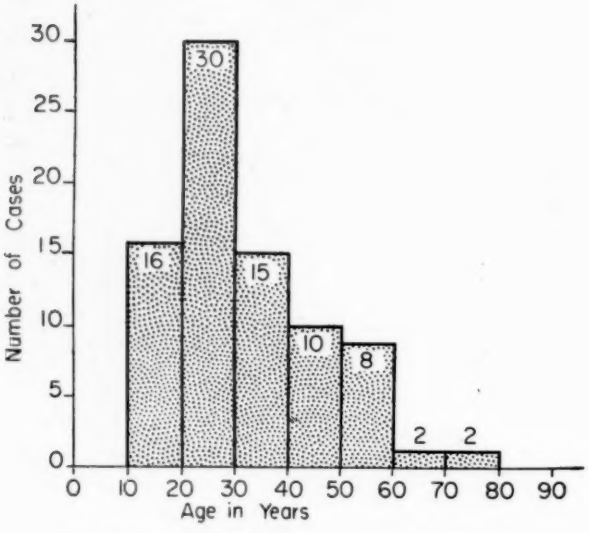

FIG. 2. Age distribution in eighty-three cases.

That Tietze's syndrome may have some relationship with pulmonary tuberculosis has been considered but is substantially ruled out. In the cases reviewed herein no patient was reported as having active tuberculosis; in several of the cases tuberculin skin tests were negative.

Premature calcification or ossification of the costal cartilages was thought to be associated with Tietze's syndrome by Harttung.[16] Although eight reported cases have shown calcified costal cartilages,[1,17,18,20–22] at least twenty-one have not.[3,16,23] Sufficient data are not available to compare the incidence of cartilage calcification in Tietze's syndrome with that in the general population.[24] It is, however, apparent that calcification is not a *sine qua non* for the occurrence of this syndrome.

In four reported cases there was an associated rheumatic or arthritic condition.[14,20,25] This association would appear coincidental. Although involvement of the manubriosternal[26] and chondrosternal articulations may be seen in rheumatoid spondylitis, Tietze's syndrome does not primarily involve these joints. Congenitally bifid ribs have been reported in two cases of Tietze's syndrome.[5,27] Their significance is unknown.

Precipitating factors: Hormonal factors have been suspected[28] but have not been proved[23] to have etiologic import. External trauma has been mentioned as a precipitating factor by some clinicians. Trauma to the chest appears, for example, in the histories of three of Geddes'[6] twenty-two cases, in two of Guarner's[19] three cases, and in four of Chantraine's[2] fourteen cases. Gukelberger[15] thought that "unaccustomed mechanical stress" on the cartilage was a precipitating factor in eight of his ten cases. In none of the cases verified by biopsy was a history of trauma given. The trauma of coughing has received considerable attention in the literature on this syndrome.[4,15,20,27,29] Rib fractures due to coughing are reported from time to time,[11,30–32] and violent coughing can inflict considerable stress on the costal cartilages and costochondral junctions.[11] However, in the majority of reported cases a history of cough was not given.

In a handful of cases[23,27] an association with a distant focus of infection has been described. Acute viral and bacterial infections of the respiratory tract are sometimes associated with Tietze's syndrome. All of Geddes'[6] twenty-two patients had a respiratory infection from one month before to two days after the onset of pain in the chest due to Tietze's syndrome. In the majority of cases the symptoms were those of the common cold. On the other hand, all of Düben's[3] ten patients were said to be free of disease of the respiratory tract. A statement as to the presence or absence of respiratory infections was found in sixty-five cases. Of these, fifty-one patients had either a cough or a respiratory infection; fourteen patients did not. Common colds, bronchitis and pneumonia were mentioned most frequently. This association appears to be significant but is not invariable; whether it is due to toxic factors, to the trauma of coughing or both is unknown.

The most recent etiologic theories have been proposed by American observers. Motulsky and Rohn[4] state that the anatomic location of an inconstant structure, the interarticular sternocostal ligament, parallels the incidence of involvement of the various cartilages in Tietze's

syndrome. They suggest that "microtrauma" or disease of this ligament may be a causative factor. Beck and Berkheiser[5] have proposed that the pathologic process consists of a contracture of the ligament lying subjacent to the second costal cartilage, causing the cartilage to buckle forward.

The possibility of multiple etiology is mentioned by Raffle,[27] who believes that such factors as malnutrition, infection of the respiratory tract and local congenital anomalies could all be operative in the same individual. He believed the essential factor to be an "unusual distribution of local stresses and strains" on the cartilages.

Epidemiology. All cases reported have been isolated with the possible exception of Geddes'[6] series. Although he reported four patients from one regiment, three from one reinforcement unit and two from one transport unit, he does not imply that contagion was involved.

The season of onset was given in eight of the biopsied cases. Of these, five cases appeared in winter,[23,25,33,34] one in "fall or winter"[6] and two in summer.[16,20] As to geographic distribution, biopsied cases have been reported from six Western European countries, Japan and the Eastern United States.

Pathology. The site of the lesion was stated in ninety-six cases. Of these, sixty-six patients (68.75 per cent) had single swellings; thirty patients (31.25 per cent) had multiple swellings. Of the sixty-six swellings, equal numbers were right- and left-sided. The single lesions were localized as follows: first costal cartilage, five; second, forty-six; third, six; fourth, six; seventh, two; tenth, one. When the swellings were multiple, neighboring cartilages on the same side were most often involved. Only two patients[6,15] had bilateral lesions. Thirteen patients presented two lesions; twelve patients, three lesions; and three patients, four lesions. One case each of five and six swellings is reported.

Of the cases in which biopsy specimens were taken, the site of the lesion was stated in thirteen. Nine patients presented single swellings; in four they were multiple. The locus of swellings in these thirteen cases is diagrammed in Figure 3.

Gross findings at operation have been difficult to interpret. (Table I.) Some observers have noted swelling or edema of the perichondrium and soft tissues; others have not. The adjoining ribs have appeared normal except in three cases.[21] To most authors the cartilage itself appears abnormally prominent. It is not clear whether this represents an actual increase in the mass of the cartilage or simply a forward angulation. Beck and Berkheiser[5] take the latter view.

No specific histologic changes have been found in Tietze's syndrome. (Table I.) Typically, the report is of essentially normal cartilage. No signs of tumor or inflammation were found except in one case[35] in which the findings were compatible with a granuloma.

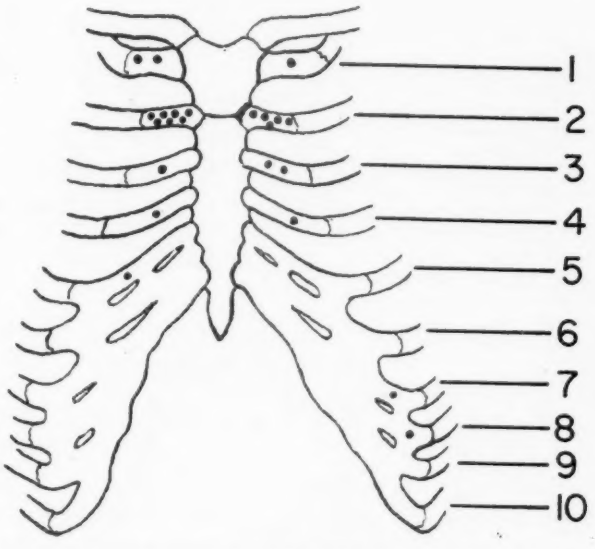

FIG. 3. Location of lesions (indicated by dots) in thirteen cases in which biopsy was performed.

Clinical Manifestations. The chief complaint in the vast majority of reported cases was pain, although an occasional patient noted a swelling before the onset of pain. Pain is generally localized to the affected cartilage, but radiation laterally and even into the arm has been described.[3,34] Onset is sometimes sudden[6] and sometimes gradual. Intensity ranges from mild to severe. Patients have described the pain variously as aching, gripping, neuralgic, sharp, dull, and even as "gas pains." Exacerbation with coughing or deep breathing has been reported.[3,36]

On physical examination the finding of a firm, fusiform or spindle-shaped swelling confined to one or more of the upper four costal cartilages is characteristic. Heat and erythema are absent. Tenderness is usually although not always present initially; when present it seems to be the first finding to disappear. Almost all investigators have found the tenderness to disappear in ten days to eight or nine weeks.

TABLE I

ROENTGENOLOGIC AND PATHOLOGIC FINDINGS IN TWENTY-FOUR BIOPSIED CASES

Author	No. of Cases	Age (yr.) and Sex	X-ray Findings	Pathologic Findings	
				Gross	Microscopic
Tietze[1]	1	18, M	No data	Second costal cartilage peculiarly bulged forward; cartilage not altered externally; overlying soft tissues, perichondrium, muscle, fasciae and ligaments were thickened	Irregular arrangement of cartilage suggests the structure of tumor; no evidence of malignancy or inflammation; occasional calcareous deposits with splitting into fibers (Auffaserung)
Breslauer Chirurgische Gesellschaft[35]	1	26, F	No data	No data	No blastomatous infiltration; in the attached soft parts were nodules of fibroblasts, 1 of which consisted in part of an enormous number of giant cells; diagnosis was simple granulating inflammation
Harttung[16]	1	24, M	No shadows indicating calcification of cartilage	Cartilage perhaps somewhat pale	Calcareous deposits of various sizes; atrophic processes; degeneration of muscle cells; complete wasting; fibrillation (Auffaserung) of the ground substance as well as formation of fibrous tissue at 1 site
Himei[33]	1	20, M	Affected cartilage widened; otherwise normal	No necrosis; no change in consistency or color of cartilage; slight thickening toward middle of cartilage	Normal structures with no sign of inflammation or regressive changes
Satani and Fujii[17]	1	?, F	No data	No granulation or pus; cartilage not distinctly altered	Fibrotic changes, ossification and atrophy of cartilage
Gill, Jones, and Pollack[14]	1	29, M	Soft tissue swelling; no bony abnormality	Spindle-shaped enlargement of cartilage; intercostal tissues edematous; cartilage appeared normal on cut section; opaque streaks	Typical cartilaginous matrix; cells flattened and small along 1 border; groups of 6 to 8 cells frequent; a few rather wide blood spaces and more abundant smaller ones were present, also areas having a fibrillar structure; fibers stained deeply with Van Gieson's stain, so presumed to be collagen; no evidence of inflammation; diagnosis was either normal cartilage or chondroma
Geddes[6]	1	?, M	No data	Cartilage and perichondrium swollen; no swelling of other contiguous structures	Appearance does not suggest true neoplasm; shows hypertrophy of cartilage, some of which is fibrocartilage, particularly on external aspect; in a few areas there is metaplasia of cartilage with early bone formation; in these areas there is an increase in vascularity; in other areas there is some calcification; in the bony portion there is fibrosis of marrow; perichondrium thickened due to an increase in fibrous tissue; some areas suggest atypical callus formation; does not appear to be simple chondroma
Leger and Moinnereau[21]	4	.....	Increase in size and porosity of anterior extremity of rib; mottled appearance and few scattered calcific deposits; soft tissue swelling; x-rays in 2 cases normal	In 3 cases adjacent rib showed a bluish devitalized (délavé) appearance	Cartilage normal or hyperplastic; some cases showed areas of osseous metaplasia, increased vascularity and disseminated calcareous deposits; marrow fibrosis of adjacent rib seen in 1 case; "nothing abnormal" in 2 cases; no signs of inflammation or malignancy in any case
Pasquali[34]	1	32, F	Ribs normal	Cartilage swollen for 3 cm. near costochondral junction; perichondrium opaque and rather thickened; no gross changes of appearance or constistency of cartilage itself	Cartilaginous tissue normal except a zone in which the normal arrangement of the intercellular substance in spots was altered, and slight regressive phenomena of cellular elements were seen
Bruin and Smook[25]	1	55, F	No data	No data	No particular abnormalities considering the patient's age
Laake[22]	1	22, F	No radiopaque areas in costal cartilages; skeletal survey normal	Perichondrium slightly thickened	Normal hyaline cartilage structure

TABLE 1 (*Continued*)

Author	No. of Cases	Age (yr.) and Sex	X-ray Findings	Pathologic Findings	
				Gross	Microscopic
De Haas[20]	1	57, F	Fairly marked calcification of costal cartilages	No data	No real abnormality
Chantraine[2]	1	37, F	Negative	No data	Typical cartilaginous tissue with orderly cell arrangement; some centers of calcification, nothing abnormal at the costochondral junction
Düben[3]	3	?, F	Normal bony anatomy without destruction or periosteal reaction; except for the almost constant physiologic calcification of first costal cartilage after age 30, no calcific deposits seen	No gross changes of bone, periosteum, cartilage or perichondrium; rib synchondroses showed forward angulation to a greater than normal degree; the rib itself was somewhat bent in the same direction	Normal conditions throughout, both in bony and cartilaginous parts of ribs; inflammation and neoplasm could be ruled out with reasonable certainty
Beck and Berkheiser[5]	4	22, F; 34, F; 11, M	Entirely negative in all patients	Cartilage approximately normal in size and thickness; it was buckled forward in an acute angle; immediately subjacent to the cartilage heavy ligamentous fibrous tissue bands visible; bifid rib in 1 case	Removed cartilages entirely normal
Kayser	1	16, F	Nothing abnormal in thoracic wall; costal cartilage normally radiolucent and showed no calcification	No evidence of adhesions or inflammation in overlying tissues; moderate degree of anterior angulation of second right costal cartilage at costochondral junction; perichondrium smooth, not grossly thickened; cartilage appeared normal on section	Normal cartilage and bone; no evidence of tumor or inflammation; adjacent bone showed numerous cement lines, suggesting waves of bone formation.

Spontaneous pain usually persists longer than tenderness. Although in some cases pain lasted only one week, it is usually described as lasting a few weeks to several months. Gradual subsidence over a period of weeks was the most frequent finding, with exacerbations not uncommon.

The palpable swellings seem to reach a maximum size, then either remain stationary or slowly regress. Exacerbations and remissions of the swellings have been claimed. Very few of these lesions have been observed to undergo complete regression. Some cases have been followed up in which the swelling was present sixteen to twenty-six months after onset.[20,23,25,33] A few patients have given histories of such swellings being present for three to five years.[2,5] A prolonged course is the rule.

In most cases no roentgenographic abnormalities are reported. (Table 1.) Lesions have occurred in cartilages which showed calcific deposits and in those which did not. Routine laboratory work, including complete blood count, urinalysis, sedimentation rate and serologic tests for syphilis, has been reported as normal in several cases. De Haas[20] found the blood calcium, phosphorus and alkaline phosphatase levels to be normal in two patients. The total cholesterol and uric acid levels of these patients were "on the high side." Laake[23] found normal urinary excretion of estrone and follicle-stimulating hormone in four young women with Tietze's syndrome.

Diagnosis and Differential Diagnosis. The rather characteristic clinical picture should suggest the diagnosis. Confirmation is largely a matter of exclusion. Involvement of the costal cartilage by systemic disease should be ruled out. Positive diagnosis is made when a biopsy specimen showing essentially normal costal cartilage is taken from a patient with the typical clinical findings. Needle biopsy of these lesions has proved unsuccessful. Surgical biopsy is necessary to rule out the many specific lesions which may cause swellings of or about the costal cartilages. A partial list of such lesions would include both benign and malignant neoplasms, e.g., chondroma, osteochondroma, multiple myeloma, osteogenic sarcoma, Ewing's tumor, Hodgkin's disease and

metastatic tumors, especially carcinoma of the breast and lung.[3,4,9,37,38]

Many specific infections of costal cartilage have been described. Pyogenic organisms may give rise to chronic osteomyelitis of the nearby rib or to metastatic bacterial osteochondritis from a focus of infection elsewhere in the body.[3,29,34,39,40] Lesions of costal cartilage have been described in tuberculosis, syphilis,[6,34,41] typhoid and paratyphoid infections,[3,40] blastomycosis, actinomycosis[6,40] and brucellosis.[29,34]

Rheumatoid arthritis of the second to fifth chondrosternal joints, costochondral separation, postfracture callus formation and other rarer entities have been mentioned as possibilities in the differential diagnosis.[4,11,23]

Treatment. There is no specific treatment. Symptomatic measures which have been recommended include reassurance and analgesics,[3,4,17,41] rest and local heat,[13] short wave therapy,[3,15] and local counterirritants.[15] Roentgen radiation was used by Laake[23] "without convincing effect," and Chantraine[2] found a local dose of 1,400 r to be ineffective in one case.

Partial or complete resection of the involved cartilage has been performed in refractory cases. Beck and Berkheiser[5] excised the involved cartilages in four patients, leaving behind a cuff of perichondrium to insure healing. They reported no recurrences following operation.

DISCUSSION

The etiology and pathogenesis of Tietze's syndrome remain unknown. The syndrome even defies positive inclusion in one of the known categories of disease: infectious, neoplastic, metabolic, degenerative, traumatic, etc. Indeed, it may be a disease, not of cartilage at all but of the surrounding ligaments.[4,5]

The question may arise as to whether these lesions are not simply painful chondromas, since histologic distinction between chondroma and normal cartilage is sometimes difficult. Several differences are apparent: The typical chondroma is lobulated, whereas no lobulations have been reported in Tietze's syndrome; in the majority of chondromas of the chest wall, pain is a late symptom, if it appears at all;[37] chondromas of the chest wall have no preference for upper or lower ribs; whereas Tietze's lesions show a predilection for the upper ribs, especially the second. The chondroma grows steadily and may reach enormous proportions, whereas the lesion of Tietze's syndrome appears to limit itself to slight or moderate swelling which is capable of spontaneous regression.

Tietze's syndrome is of clinical interest primarily because of its significance in the differential diagnosis of chest pain. The pain of Tietze's syndrome may mimic that of pleurisy, angina pectoris, intercostal neuritis and other more common syndromes.

A British physician in India[42] candidly reported that a patient with pain in the chest first noticed the "lump" on his chest while the doctor was examining his back. It follows that a history of pain in the chest should lead to inspection and palpation of the thoracic wall, as well as auscultation of the heart and lungs.

SUMMARY

Tietze's syndrome consists of an initially painful, usually tender prominence of one or more of the upper costal cartilages for which no specific etiology can be found. Biopsy usually shows normal cartilage. The clinical course is benign but may be prolonged.

A case of this syndrome is presented and the findings in 159 cases from the literature are analyzed, with emphasis on the biopsied cases.

Acknowledgments: The author is deeply grateful to Arthur M. Rogers, M.D., formerly Associate Professor of Clinical Medicine, The University of Pennsylvania School of Medicine. His clinical skill was responsible for making the diagnosis in the case reported herein. Doctors René Lefevre, Masashiro Sakai and Carlos Roblés generously did many of the necessary translations.

ADDENDUM

The writer inadvertently omitted from this study a careful pathologic description of a resected specimen of the second left costal cartilage from a twenty-three year old man. The painful swelling in this case was due to a persistent fracture of the adjacent rib with subsequent callus production and the formation of pseudarthrosis. (Wepler, W. Ueber die sog. Tietzesche Krankheit. *Deutsche med. Wchnschr.*, 79: 137–139, 1954.)

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Seizures in Infectious Mononucleosis*

With a Review of the Literature

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NEUROLOGIC manifestations of infectious mononucleosis have been reported with increasing frequency in recent years. Leibowitz, in his comprehensive monograph in 1953, compiled a group of seventy-one cases of neurologic complications fulfilling the criteria for infectious mononucleosis and since that publication authenticated cases have appeared with regularity.

Though the earliest cases were considered to be serous meningitis,[1,2] one analysis would have us classify by symptom into serous meningitis, encephalitis, myelitis, neuritis and convulsive states.[3] We have recently observed a case of infectious mononucleosis presenting with a seizure followed by high fever and stupor and we were prompted to investigate the incidence of convulsive episodes in this disorder.

REVIEW OF THE LITERATURE

Infectious mononucleosis has long been known to be a disease of protean manifestations, though the usual conception is that of an enervating affliction of college students and young adults. Considered probably to be infectious and of virus etiology, the mode of transmission has never been established, but oral contact has been strongly suspected.[4,5]

Sir Henry Tidy distinguishes six clinical groups of glandular fever: (1) juvenile glandular type with rapid formation of a visible mass of glands; (2) adolescent type—more insidious and commoner in young adults; (3) prolonged febrile types; (4) anginose type (monocytic angina); (5) with neurologic manifestations; and (6) onset with jaundice.[6] The incidence of neurologic complications has been estimated as less than one per cent by Bernstein and Wolff[7] and 0.37 per cent by Silversides and Richardson.[3] In the seventy-one cases accumulated by Leibowitz there were eight deaths, an incidence of 11 per cent, at once indicating a more guarded prognosis in cases of this sort, with respiratory paralysis the cause of death in the five cases in which autopsies were performed.[8] The pathologic changes have been described by several observers as characterized by selective degeneration of nerve cells, hemorrhages, congestion and perivascular cuffing.[9]

The association of seizures and infectious mononucleosis is uncommon, and we have been able to collect and analyze only thirteen such cases, including that of Ricker et al. in which the convulsion was a terminal event.[10] The salient features are to be found in Table 1. Hubler et al. in 1951 were able to collect only six cases in which convulsions were observed.[14] The seizures appeared to have no distinctive pattern but rather were symptomatic of generalized cerebral involvement. Varying periods of stupor or unconsciousness followed and incontinence occurred in three cases. The youngest patient was eight years of age, the oldest twenty-four. Except for the fatal case reported by Ricker et al. recovery was the rule, therefore one might suggest that the supervention of seizures is not a poor prognostic sign. Although a pleocytosis was not invariable, increased spinal fluid protein seemed to be fairly constant. In two cases the spinal fluid heterophil antibody titer was found to be positive and the blood heterophil ranged from 1:448 to 1:2048.

Slade in 1946 was the first to report the use of the electroencephalogram in infectious mononucleosis.[19] In the present series abnormal records were noted in four cases. Bercel has been active in investigating the form of the electro-

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TABLE I

SEIZURES IN INFECTIOUS MONONUCLEOSIS

Report	Age, Sex	Signs and Symptoms	Heterophil Antibody Titer	Seizures	Cerebrospinal Fluid	Recovery
Sucher, Schwarz[11]	17,F	Generalized nodes; splenomegaly; hepatomegaly; typical blood picture	Not done	Confusion, convulsions, opisthotonus and status epilepticus 1 mo. after onset, then left hemiparesis and right oculomotor paresis	Pressure elevated; clear w. pellicle; 100 lymphocytes; Pandy, 4+; Wassermann, negative	Complete in 55 days
	15,F	Generalized nodes; splenomegaly; typical blood picture	Not done	Coma, opisthotonus and convulsions for 7 hr., then left hemiparesis	Pressure elevated; 100 lymphocytes	Complete in 14 days
Davidsohn[12]	16,F	Fever; sore throat w. ulcerative and membranous pharyngitis; generalized nodes; splenomegaly; hepatomegaly; typical blood picture	1:896	Seizure 2 wk. after onset of U.R.I., leading to unconsciousness	Clear; 18 cells	Complete
Ricker et al.[10]	22,M	Headache; fever; arthralgia; slight anterior cervical adenopathy; typical blood picture	1:1792	Sudden terminal clonic convulsion	Pressure normal; clear; 97 cells (94% lymphocytes); protein elevated; sugar normal; sterile	Death in respiratory paralysis
Field[13]	17,M	Fever; tonsillitis; 1 cervical node; typical blood picture	1:1792	Fell to floor w. incontinence; clonic convulsions w. stertorous breathing and incontinence	Clear; 248 cells w. 230 lymphocytes; protein 216; sugar 54	Complete w. some memory difficulty
Bernstein, Wolff[7]	19,M	Bifrontal headache; fatigue; fever; cervical nodes noted on 13th day; no spleen or pharyngitis; blood picture became typical	1:448, 5th day	4th day, after lumbar puncture, had generalized seizure w. opisthotonus, turning head to right, facial twitching and tongue biting, then clonic movements and incontinence; further seizures, status epilepticus, hyper-reflexia, bilateral Babinski sign and clonus	Pressure 170; clear w. 120 cells (90% lymphocytes); sugar 60; protein 156; chlorides 700; Wassermann, negative; gold curve flat	Complete in 1 mo.
Silversides, Richardson[3]	17,M	Headache; fever; adenopathy; slight pharyngitis; splenomegaly; typical blood picture	1:1280	Sudden convulsion at onset, then stupor	Clear; protein elevated; 16 cells	Discharged w. no findings on 9th day
	24,M	Headache; backache; fever; pharyngitis; lymphadenopathy; splenomegaly; typical blood picture	.....	Generalized seizure on 9th day w. no loss of consciousness; 2 more followed	Pressure 230; protein 64; 29 lymphocytes; heterophil 1:40	Complete in 6 wk.; EEG abnormal
Hubler et al.[14]	17,M	Not "up to par;" fever; generalized headache; spleen palpable after 11 days; 22% atypical lymphocytes noted after heterophil test done	1:2048, w. absorptions	Generalized seizure for 30–60 sec., w. cyanosis but no tongue biting or incontinence; became unresponsive for 45 min.; recurrance of generalized seizures after 4 days, leading to coma (began w. head turning to right)	Pressure normal; clear; 33 lymphocytes; protein 120	ACTH, dilantin and barbiturate therapy, w. complete recovery
Freedman et al.[15]	21,M	Upper respiratory infection; headache; lethargy; generalized lymphadenopathy; typical blood picture	1:1792	Twitching of left side of face w. deviation of eyes to right; generalized convulsion resulting in semi-stupor	Pressure normal; clear; 25 lymphocytes; protein 195, 154 and 474; sugar 79; heterophil 4+ w. sheep r.b.c.; quantitative titer 1:28; gold curve 541,000,000	Complete
Stride[16]	22,M	Fever; generalized nodes; 68% atypical lymphocytes; spleen not palpable	1:896, w. absorptions	Laughed, jerked head backward; tonic and clonic convulsions; bit lip and foamed at mouth; cyanosis and semi-stupor	Pressure 140; clear; protein 120 w. elevated globulin; 5 cells; sugar 70; gold curve flat	Complete
Walsh et al.[17]	8,M	Sore throat; swollen cervical nodes; fever; splenomegaly; atypical lymphocytes 3rd hosp. day	1:1024	Right-sided seizure during lumbar puncture, then frequent convulsions	Pressure 6 mm.; clear w. 10 cells (7 w.b.c.); protein 256	Complete w. slight intention tremor, finger-nose test
Natzke, Whiting[18]	24,M	Retro-orbital headache; scattered nodes; minimal pharyngeal exudate; blood picture typical	1:160 (guinea pig absorption)	Generalized tonic and clonic convulsions w. no incontinence for 30 sec. leading to semi-coma; 2nd seizure 30 min. later w. incontinence and violent motion of extremities	Pressure 110; clear; 10 cells w. 8 lymphocytes; protein 175; sugar 108	Complete

encephalogram in infectious mononucleosis and found five abnormal tracings in thirty-one cases referred because of suspicion of neurologic complications. The most common abnormality he noted was generalized high voltage slow waves concentrated in multiple foci, and he was struck by the ease with which the foci shifted from day to day without accompanying neurologic phenomena.[20] Silversides and Richardson's patient, when examined two months after the acute illness, still presented a record with a few short runs of six per second slow waves.[3] Bercel has reported two cases of post-encephalitic epilepsy following infectious mononucleosis.[21]

TABLE II

LABORATORY DATA IN A CASE OF INFECTIOUS MONONUCLEOSIS ENCEPHALITIS

Date	7/12	7/13	7/14	7/15	7/18	7/20	7/26	8/3	8/8
Temperature (maximum)	105	103	102	100	N	N	N	N	N
Blood:									
Leukocytes	6,450	13,000	8,350	4,500	12,150	5,750	5,000	. . .	9,000
Neutrophils:									
Segmented	30	22	24	35	39	47	46	...	40
Non-segmented	8	31	20	1	0	2	0	...	0
Lymphocytes	59	43	47	63	56	45	50	...	46
Monocytes	3	4	8	1	4	4	3	...	5
Eosinophils	0	0	1	0	0	2	1	...	9
Basophils	0	0	0	0	1	0	0	...	0
Hemoglobin	11.5	10.2	9.7	9.7	12.5				
ESR (Wintrobe)	18			25					
Heterophil	. . .	1:448	. . .	1:1792	1:448	1:896	1:224	0	1:56
Guinea pig absorption	. . .	1:112	. . .	1:448	1:224	1:224	1:112	. . .	1:14
Beef RBC absorption	. . .	0	. . .	0	0	0	0	...	0
Cerebrospinal fluid:									
Appearance	clear	. . .	clear	. . .	. . .	clear	. . .	...	. . .
Pressure	. .	. . .	105	. . .	. . .	. . .	. . .	...	. . .
Cells:									
Leukocytes	4	. . .	12	. . .	. . .	15	. . .	...	. . .
Lymphocytes (%)	100	. . .	50	. . .	. . .	100	. . .	...	. . .
Sugar	. . .	. . .	67	. . .	. . .	. . .	. . .	...	. . .
Protein	48	. . .	70	. . .	. . .	81.5	. . .	...	. . .
Globulin	. . .	. . .	no rise	. . .	. . .	trace	. . .	...	. . .
Heterophil	. . .	. . .	0	. . .	. . .	2 plus	. . .	...	. . .
Bacteriology	sterile	. . .	sterile	. . .	. . .	. . .	. . .	...	. . .

CASE REPORT

A nineteen year old airman who was an academic instructor was first admitted to the 3275th USAF Hospital on the morning of July 12, 1955. History was obtained from his wife, who stated that he had complained of an occipital headache for about two weeks and had taken aspirin tablets to obtain relief. She furthermore stated that the patient had had a sore throat and possibly fever for several days. On the day prior to admission the patient reported to sick call and was given "headache pills." Past history was otherwise unrevealing except for the fact that he had been hit by a car at the age of three, causing a laceration of the "side of the head."

The wife stated that the patient arose to get a drink at 3:00 A.M. on the morning of admission. Upon returning to bed he had a generalized seizure without tongue-biting or incontinence, characterized by stiffening of the body, followed by wild waving of the arms, protrusion of the lips, marked diaphoresis and heavy breathing. This subsided after about thirty minutes but commenced again in twenty minutes with arm-waving and incoherent mumbling. He fell out of bed and vomited once. The patient was brought to the emergency room in that condition, semicomatose and reacting violently to all stimuli. Here he was found to have a blood pressure of 110/70, pulse 88, respiration 36, with a temperature of 99°F. He resisted movement of the head with violent movements. Examination revealed dilated pupils, hyperactive knee jerks and an extensor plantar response on the left and possibly on the right as well. The patient required 12 grains of intravenous amobarbital in order that a lumbar puncture might be performed.

When seen in the contagion ward he was semicomatose and unresponsive except to painful stimuli, thrashing about and requiring restraints. There was no nuchal rigidity. A depression was palpable on his occiput. Neither his fundi nor his throat could be visualized. Neurologic examination revealed no motor weakness. An extensor plantar response was again elicited on the left, questionable bilateral ankle clonus was observed, and the deep tendon reflexes were equal and active. Temperature was now 101.6°F. and respirations were 38 per minute. The patient required more sedation and was given amobarbital and paraldehyde. He was given intravenous fluids and, after a blood culture was drawn, was given penicillin and streptomycin, along with aspirin administered by rectum and dilantin.® In spite of these measures the temperature rose to 105.2°F. that night with a respiratory rate of 58 per minute, the patient was given oxygen and ice-water enemas. The following morning his status had changed little and blood was drawn for viral encephalitis serologic studies. Roentgenograms of the skull were negative. Neurologic examination revealed bilateral extensor plantar responses and deep tendon reflexes were depressed throughout. A roentgenogram of the chest taken while the patient was in a recumbent position revealed bilateral pneumonitis and air was noted in the soft tissues of the neck on the right side. This was confirmed by the finding of crepitus on the right side

of the neck, and the possibility of a suppurative mediastinitis secondary to an esophageal perforation was considered.

The neurosurgeon (R. M.) who had followed the case from the outset had been disturbed by the report of 59 per cent lymphocytes with five atypical lymphocytes. This was not considered too unusual, however, since similar findings have been noted in over 800 cases of rubella seen in the contagion service. Nevertheless, the blood smear was reviewed and a heterophil antibody titer obtained, thereby establishing the diagnosis.

Forty-eight hours from the time of admission the patient had become oriented in time and space, and the neurologic examination was negative except for a depressed knee jerk on the right; pathologic reflexes were no longer present. The patient complained of intense occipital headache. Lumbar puncture was repeated and revealed normal pressure, a protein of 70 mg. per cent, and 12 cells per cu. mm., of which 50 per cent were lymphocytes. A direct heterophil antibody titer performed on this fluid was negative. By this time the patient was quite lucid and taking fluids well. The air previously noted in the soft tissues of the neck was gone. The pulmonary parenchyma revealed mottled infiltrates along with bilateral hilar adenopathy, both of which disappeared within a week. A bone marrow examination was non-diagnostic. By the fifth hospital day the temperature was normal. There was edema and a whitish cast to the uvula, attributed to previous suctioning, hyperpnea and oxygen therapy. Of the sixth hospital day the spleen was palpable for the first time, 4 cm. below the costal margin. Occipital headache was still prominent.

On the ninth hospital day, lumbar puncture was again performed, revealing fifteen lymphocytes and 81 mg. per cent protein. Examination of the spinal fluid for heterophil antibody by the method of Silberstein et al.,[22] utilizing 1 ml. of cerebrospinal fluid, was reported as 2 plus. By the tenth hospital day headache had vanished, but splenomegaly without significant lymphadenopathy was still present and persisted one month after admission. There was a weight loss of 23 pounds during the early part of the illness but this was gradually regained. Liver function tests during the second week of hospitalization revealed normal bromsulphalein retention, thymol turbidity, alkaline phosphatase, cholesterol and esters, bilirubin and prothrombin time. The cephalin flocculation test was 3 plus, the serum albumin was 3.9 gm. and the serum globulin 3.6 gm. per 100 cc. One week later these findings were 2 plus, 3.8, 3.8, respectively. By one month the cephalin flocculation test was reported as trace. Complement fixation tests were reported as negative for herpes simplex, eastern and western equine encephalitis, mumps encephalitis, St. Louis encephalitis, and lymphocytic choriomeningitis. Skin tests, including PPD No. 1 and intermediate strengths, coccidiodin 1:100, and histoplasmin 1:100, were all negative. Two electrocardiograms were normal. Electroencephalograms were obtained on the fourth, eighth and twenty-ninth hospital days. The first record revealed a generally low voltage with some bilateral occipital 3 to 4 per second activity and occasional 3 to 4 per second activity in mid-parietal leads. There was no well formed alpha pattern. The record was interpreted as compatible with diffuse cerebral dysfunction. The second record revealed minimal diffuse slowing on hyperventilation, and the final tracing was normal.

In reviewing the peripheral blood smears in this case, it is interesting to note that there were between 24 and 28 per cent atypical lymphocytes in the first four differential counts. The pertinent serial laboratory findings are contained in Table II.

COMMENT

It is immediately apparent that the timing of seizures during the course of infectious mononucleosis may be quite variable, depending, of course, upon the stage of the illness during which the patient comes under medical surveillance. Although central nervous symptoms may appear as early as two weeks before lymphadenopathy and splenomegaly,[20,23] in our case the correct diagnosis might have been suspected two weeks preceding the seizure.

Our experience emphasizes the importance of absorption studies, as stressed by Leibowitz, since our first heterophil antibody titer of 1:448 was nevertheless accompanied by a characteristic absorption pattern.[24] The liver chemistry tests revealed mild abnormalities often encountered in infectious mononucleosis, with a slight increase in globulin and a three-plus cephalin flocculation test. Finally, the roentgenogram of the chest in our case revealed mottled parenchymal infiltrates and bilateral hilar lymphadenopathy. McCort, in seven cases of clinically proved infectious mononucleosis of forty-three in which roentgenograms of the chest were taken found enlargement of the mediastinal lymph nodes in two cases, both enlargement of the mediastinal lymph nodes and pulmonary parenchymal changes in three cases and parenchymal changes alone in two cases.[25]

It is worthwhile to reemphasize that infectious mononucleosis should always be suspected when the diagnosis of an encephalitis of an indeterminate type is being considered.[14,17]

SUMMARY

The association of seizures with infectious mononucleosis is an infrequent occurrence. A

review of the literature reveals no distinctive pattern of the convulsive episodes.

An illustrative case is presented with noteworthy electroencephalographic, hematologic, serologic and roentgenographic findings. Infectious mononucleosis should be considered in the differential diagnosis of unexplained and ill-defined convulsive episodes.

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(ab.) = Abstracts; (CPC) = Clinico-pathologic Conference; (E.) = Editorial

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